MANUAL OF METHODS

OF

ANALYSIS OF FOODS

CEREAL AND CEREAL PRODUCTS

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Note: The test methods described in this manual are internationally/nationally validated methods. However, it is the responsibility of the testing laboratory to verify the performance of these methods in their laboratory to meet the needs of the given application.

	Determination of Foreign Matter in Food Grains		
Method No.	FSSAI 03.001:2022	**Revision No. & Date**	0.0
Scope	The method is a gravimetric method and applicable to wheat, maize, jowar, unprocessed whole raw pulses, oats, finger millet, Whole and decorticated pearl millet grains (Bajra), chia seeds, Whole or shelled (de-husked) or split pulses: Lentil (Masur) - *Lenil esculenta* Moench or *Lens culinaris* Medik or Ervem lens Linn; Black gram (Urd) – *Phaseolus mungo* Linn; Green gram (Moong) - *Phaseolus aureus* Roxb., *Phaseolus radiatus* Roxb; Bengal gram (Chana or Chick pea) or Kabuli chana or Chole or (green chick pea) hara chana - *Cicer arietinum* Linn; Horse gram (Kulthi) –*Dolichos biflorus*; Field bean (Black, Brown, White), Sem - *Phaseolus vulgaris*; Peas dry (Matra) –*Pisum sativum*; Soybean – *Glycine max* Merr.); Rajmah or Double beans or Broad beans or Black beans –*Phaseolus vulgaris* Lobia or black-eyed beans or black eyed white Lobia – *Vigna catjang*); Moth bean (Matki) – *Phaseolus aconitifolius* Jacq.		
Caution	None		
Principle	The foreign matter is strained by using sieves of various mesh and weighed in analytical balance		
Definitions of Foreign Matter	"Foreign matter" means any extraneous matter other than food grains comprising of- (i) inorganic matter consisting or metallic pieces, sand, gravel, dirt, pebbles, stones, lumps of earth, clay and mud, animal filth and in the case of rice, kernels or pieces of kernels, if any, having mud sticking on the surface of the rice, and (ii) organic matter consisting of husk, straws, weed seeds and other inedible grains and also paddy in the case of rice.		

<table>
<tr><td>Apparatus/ Instruments</td><td>a) Analytical Balance – sensitivity 0.001 g
b) Test sieves: Wire cloth test sieves (IS 460 Part 1 1985 Third Revision). I. S sieves of round holes having following aperture size:
<table><tr><td></td><td>IS sieve</td></tr><tr><td>Top</td><td>4.0 mm</td></tr><tr><td>Second from top</td><td>3.35 mm</td></tr><tr><td>Third from top</td><td>1.70 mm</td></tr><tr><td>Fourth from top</td><td>1.0 mm</td></tr></table>c) A solid bottom pan at the bottom
d) Enameled Trays – Flat type 30 cm diameter with raised rims
e) Small scoop
f) Forceps
g) Magnifying glass with a handle of about 7.5 cm and a magnification of 10×.</td></tr>
<tr><td>Method of analysis</td><td>1. Accurately weigh 500 g of the grain and record mass of the sample.
2. Pour the quantity over the set of sieves previously arranged in such a way that the sieve with the largest perforation is at the top and those with smaller perforations are placed in the descending order of their sizes and the solid pan at the bottom.
3. Agitate the sample thoroughly to strain out the foreign matter at various levels.
4. As a result of this straining, other food grains and foreign matter like bold pieces of clay, chaff etc. shall remain on the first three sieves according to their sizes.
5. The top most sieve would contain bold grains, big pieces of clay and other big sized foreign matter, while the lower sieves would contain smaller, shriveled and badly insect damaged grains and smaller foreign matter.
6. Separate the sieves after straining and pick up all foreign matter by hand or with tweezers and add it to the foreign matter collected on the bottom pan.
7. Weigh the total foreign matter of the bottom pan and calculate the percentage.
8. Report the figure so obtained as the percentage of foreign matter in the food grain

In the case of rice, millets and smaller sized grains the quantity of sample for test should be 250 g.</td></tr>
</table>

	For the purpose of reducing the quantity of test sample, spread the entire sample in a tray, divide it into four equal portions, collect two opposite quarters and repeat this process till the required quantity of sample is collected.
Calculation and units of expression	$$\text{Foreign matter } (\%) = \frac{\text{Mass of extraneous matter}}{\text{Mass of sample}} \times 100$$
Reference	1. AOAC 17th edn, 2000, Official method 970. 66 Light and Heavy Filth 2. IS 4333 (Part 1): 1996 Methods of analysis for Food grains Part I Refractions
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Mineral Matter		
Method No.	FSSAI 03.002:2022	**Revision No. & Date**	0.0
Scope	The method is a gravimetric method and applicable to wheat, maize, jowar, unprocessed whole raw pulses, oats, finger millet, Whole and decorticated pearl millet grains (Bajra), Chia (Salvia hispanica L) seeds, Amarant (*Amaranthus caudatus, Amaranthus cruentus and Amaranthus hypochondriacus*) Whole or shelled (de-husked) or split pulses as listed in 2.4.6.22 of Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011 Lentil (Masur) - *Lens esculenta* Moench or *Lens culinaris* Medik or Ervem lens Linn; Black gram (Urd) – *Phaseolus mungo* Linn; Green gram (Moong) - *Phaseolus aureus* Roxb., *Phaseolus radiatus* Roxb; Bengal gram (Chana or Chick pea) or Kabuli chana or Chhole or (green chick pea) hara chana - *Cicer arietinum* Linn; Horse gram (Kulthi) –*Dolichos biflorus*; Field bean (Black, Brown, White), Sem - *Phaseolus vulgaris*; Peas dry (Matra) –*Pisum sativum*; Soybean – *Glycine max* Merr.); Rajmah or Double beans or Broad beans or Black beans – *Phaseolus vulgaris* Lobia or black-eyed beans or black eyed white lobia – *Vigna catjang*); Moth bean (matki) – *Phaseolus aconitifolius* Jacq.		
Caution	Carbon tetrachloride: Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Wear protective gloves/ protective clothing/ eye protection/ face protection and use fume hood. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.		
Principle	The mineral (inorganic) is determined by dissolving the organic matter in Carbon tetrachloride.		
Apparatus	Analytical balance: Accuracy 0.01 g		
Materials and Reagents	Carbon tetrachloride		

Method of analysis	1. Transfer the entire foreign matter collected from previous procedure into a beaker containing carbon tetrachloride. 2. Allow the inorganic extraneous matter (mineral matter) to settle down and organic matter is dissolved. 3. Filter or decant the solution. 4. Dry the residue at 100 °C and weigh. 5. Calculate the percentage. The remaining amount shall be the mineral matter.
Calculation with units of expression	Inorganic matter (%) $=\frac{Mass\ of\ inorganic\ residue}{Mass\ of\ sample} \times 100$
Reference	1. AOAC 17th edn, 2000, Official method 970. 66 Light and Heavy Filth 2. IS 4333 (Part 1): 1996 Methods of analysis for Food grains Part I Refractions
Approved by	Scientific Panel on Methods of Sampling and Analysis

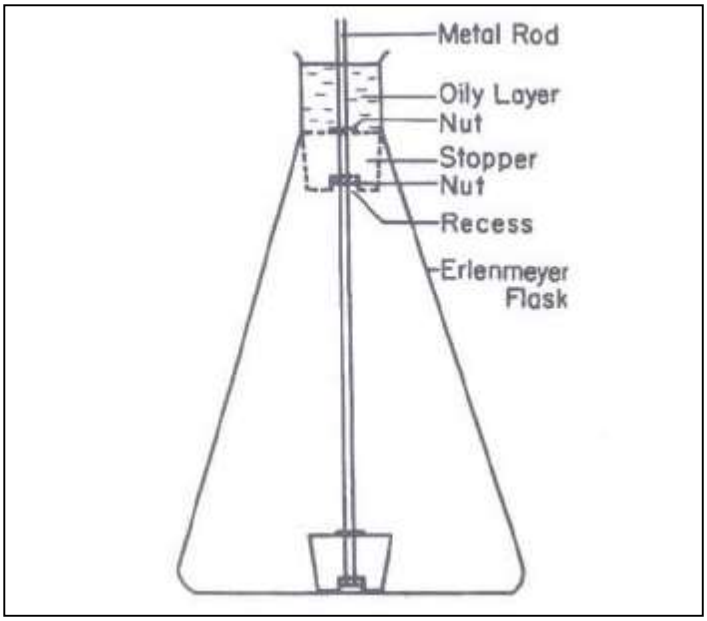
	Determination of 1) Refraction other than Foreign Matter and 2) Insect Damaged Grains		
Method No.	FSSAI 03.003:2022	**Revision No. & Date**	0.0
Scope	The method for the determination of refractions in food grains is described. The method is applicable to all food grains listed under 2.4.6 of the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 and amendments thereof. The definitions for the refractions are as described in the regulations.		
Principle	The procedure is based on visual examination and collection of the refractions and determining the mass fraction of each refraction either percent by mass or percent by count.		
Apparatus/Instruments	a) Balance – Sensitivity 0.001 g b) Test sieves: Wire cloth test sieves (IS460 Part 1 1985 Third Revision). I.S sieves of round holes having following aperture size: IS sieve Top 4.0 mm Second from top 3.35 mm Third from top 1.70 mm Fourth from top 1.0 mm c) A solid bottom pan at the bottom d) Enameled Trays – Flat type 30 cm diameter with raised rims e) Small scoop f) Forceps g) Magnifying glass with a handle of about 7.5 cm and a magnification of 10×.		
Materials and Reagents	None		
Definitions for refractions	The definition for various refractions given under 'Explanation" in 2.4.6.15 for items 2.4.6 (2-14) in Food Safety and Standards and Food Additives) Regulations, 2011 are: *Karnal bunt* – Grains of wheat having a dull appearance and blackish in colour, the blackness spreading along the longitudinal furrow on the ventral side giving the kernels a boat like appearance. The grains are affected by a field fungus *Neovossia indica.* *Ergot* – Grains of wheat showing a slightly curved body in the ear in place of kernel. Ergot is produced by fungus *Claviceps*		

	pupurea. Ergot produces Ergo toxin and occurs in rye, millets and wheat. (Ref: - I.S: 8184 – 1976 Method for determination of Ergot in Food grains). *Foreign matter* means any extraneous matter other than food grains comprising of- I. inorganic matter consisting or metallic pieces, sand, gravel, dirt, pebbles, stones, lumps of earth, clay and mud, animal filth and in the case of rice, kernels or pieces of kernels, if any, having mud sticking on the surface of the rice, and II. organic matter consisting of husk, straws, weed seeds and other inedible grains and also paddy in the case of rice; *Poisonous, toxic and/or harmful seeds* - means any seed which is present in quantities above permissible limit may have damaging or dangerous effect on health, organoleptic properties or technological performance such as Dhatura (*D. fastur linn and D. stramonium linn*), corn cokle (*Agrostemma githago L, Machai Lallium remulenum* linn), Akra (*Vicia* species). *Damaged grains*-means kernels or pieces of kernels that are sprouted or internally damaged as a result of heat, microbe, moisture or whether, viz., ergot affected grain and kernel bunt grains; *Weevilled grains*-means kernels that are partially or wholly bored by insects injurious to grains but does not include germ eaten grains and egg spotted grains; *Other edible grains*-means any edible grains (including oil seeds) other than the one which is under consideration. *Heat-Damaged*-means kernels, whole or broken, that have changed their normal colour as a result of heating; *Damaged Kernels*-means kernels, whole or broken, showing obvious deterioration due to moisture, pests, diseases, or other causes, but excluding heat-damaged kernels; *Immature Kernels*-are unripe or undeveloped whole or broken kernels; *Chalky Kernels*-means whole or broken kernels except for glutinous rice, of which at least three quarters of the surface has an opaque and floury appearance; Kernels with Pinpoint-are kernels or pieces of kernels having minute black spot of pin point size.

<table>
<tr>
<td>Method of Analysis for refractions other than insect damage grains</td>
<td>
1. Mix the contents of the four sieves freed from foreign matter together and spread out evenly on a flat smooth surface.
2. From this spread, take exactly the specified quantity required for analysis as indicated below from different parts by quartering the sample.
<table>
<tr><td>Bolder grains such as: Wheat/Maize/Barley/Whole pulses</td><td>50 g</td></tr>
<tr><td>Smaller grains such as: Rice/Split pulses/millets</td><td>20 g</td></tr>
</table>
3. Place the weighed quantity in an enameled tray. Then pick out by hand with the help of magnifying glass, if necessary, various refractions as per the definitions given in Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. Pick the refractions in the order given below, care being taken that each refraction is accounted for only once.
I. Other food grains
II. Damaged
III. Discolored
IV. Insect damaged
V. Fragments
VI. Broken
VII. Slightly damaged or touched
VIII. Chalky (in case of rice)
IX. Red grains
X. Kernels with husk
XI. Shriveled or immature
XII. Varietal admixture
4. Separate the refractions from the weighed sample and weigh on the physical balance.
5. Calculate the percentage of various individual refractions separately on the quantity taken for actual analysis
</td>
</tr>
<tr>
<td>Calculation with units of expression</td>
<td>$$Refraction\ (\%) = \frac{Mass\ of\ refraction}{Mass\ of\ grain\ taken} \times 100$$</td>
</tr>
<tr>
<td>Method of Analysis for insect damaged (weevilled) grains</td>
<td>
From out of the sieved sample free from foreign matter measure 20 mL of the representative sample with the help of a measuring cylinder.
Place the measured sample on a sample plate and count the total number of grain-kernels.
Pick out the weevilled grains separately and count.
</td>
</tr>
</table>

Calculation	The insect damaged grains present in the sample shall be calculated as follows: $Insect\ damaged\ (\%\ by\ count\ ) = \frac{\text{Number of weeviled grains in 20 mL}}{Total\ number\ of\ grains\ in\ 20\ mL} \times 100$
Reference	IS 4333 (Part 1): 1996 Methods of analysis for Food grains Part I Refractions ((Reaffirmed - 2012)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method for Determination of Light Filth in Whole Wheat Flour		
Method No.	FSSAI 03.004:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to whole wheat flour (atta), refined flour.		
Caution	*Hydrochloric acid*: Handle with extreme care. Concentrated HCl is corrosive. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood. *Hot plate*: Use insulated gloves when removing containers from hot plate.		
Principle	A test portion is digested by boiling in a 3% HCl solution, and the mixture is sieved. The residue is defatted by boiling in isopropanol and the mixture is sieved again. The filth is trapped with mineral oil in a mixture of Tween 80 and Na_4EDTA in 40% isopropanol. The oil phase is trapped off, filtered and examined microscopically for filth elements.		
Apparatus/Instruments	1. Sieve: No 230 Plain weave. Plain weave is woven with one wire alternately over and under next – 2. Sieve Handle for holding 8-inch diameter sieve 3. Reflux apparatus (Solvent saver apparatus) 4. Wildman trap flask – see figure below 5. Filter paper – Ruled - Use smooth, high wet strength filter paper ruled with oil, - alcohol, and water- proof lines 5 mm apart. Whatman Grade 8 Ruled Filter Papers (White filter paper with printed green lines for optical assessment) or equivalent is recommended. 6. Magnetic stirrer with heating 7. Analytical balance: Readability 0.001 g		

	 Wildman Trap Flask Stopper on shaft is lifted up to neck of flask to trap off floating layer. Adapted from AOAC Method 945.75 Extraneous Materials in Products.
Materials and Reagents	1. Concentrated HCl 2. Isopropanol 3. Mineral oil – Paraffin oil, white, light, sp gr. 0.840 – 0. 860. Request supplier to provide certificate of analysis 4. Tween 80 (Polysorbate 80) a 5. Tetrasodium salt of Ethylenediamine tetraacetic acid (EDTA)
Preparation of Reagents	1. 3% HCl solution: Add 24 mL concentrated HCl to 776 mL water 2. 40% Isopropanol: 3. Tween 80 – 40% isopropanol solution – To 40 mL of Tween-80 add 210 mL isopropanol, mix and filter 4. EDTA–40 % isopropanol solution – Dissolve 5 g Tetrasodium EDTA in 150 mL water, add 100 mL isopropanol, mix and filter.
Sample Preparation	NA
Method of Analysis	1. Add 800 mL 3% HCl solution, C(a), to 2 L beaker. Place on preheated hot plate and magnetically stir so stirring bar is visible 2. Accurately weigh 50 g whole wheat flour to nearest 0.5 g

	into 250 mL beaker. Transfer flour portion wise to 3% HCl solution. Rinse sides of 250 mL beaker with 3% HCl solution from wash bottle and add washings to 2 L beaker. Cover with watch glass and bring to full boil. 3. Remove watch glass and boil gently 15 min with magnetic stirring. 4. Wet-sieve, and pour slurry portion wise on sieve, with gentle stream of hot (50 ° -70 °C) tap water until rinse is clear. 5. Use of sieve handle, or equivalent, is recommended. 6. Retain original beaker. 7. Wash residue to side of sieve with hot tap water, and rinse residue with 100% isopropanol. 8. Quantitatively transfer residue to original beaker, washing with 100% isopropanol. 9. Add 100% isopropanol to 400 mL mark on beaker and boil gently 5 min, using reflux apparatus, inserted into beaker top. 10. Remove beaker from reflux apparatus and quantitatively transfer beaker contents to sieve. 11. Wet-sieve with gentle stream of hot tap water until rinse is clear. Wet residue on sieve with 40% isopropanol and quantitatively transfer residue to Widman trap flask, using 40% isopropanol. 12. Dilute to 600 mL with 40% isopropanol and boil gently 5 min with magnetic stirring. 13. Remove from heat, add 65 mL mineral oil, and magnetically stir 3 min. 14. Let stand 1-2 min after stirring. 15. Add mixture of 5 mL Tween 80-40% isopropanol solution, and 5 mL Na_4EDTA-40% isopropanol solution slowly, down stirring rod. 16. Hand-stir 30 s with gentle rotary motion. Let stand undisturbed 1-2 min. 17. Fill flask with 40% isopropanol, clamp rod, and let stand 30 min. Stir bottom contents every 5 min for first 20 min and leave undisturbed for final 10 min. 18. Spin stopper (wafer) to remove any trapped residue and trap off, into 400 mL beaker, using 40% isopropanol to rinse neck of flask. 19. Add 40 mL mineral oil to flask and hand-stir 15 s with gentle up-and-down motion. 20. Fill flask with 40% isopropanol and let stand for 20 min.

	Spin stopper and trap off as before, rinsing neck with 100% isopropanol. 21. Filter beaker contents through filter and examine microscopically at ca 30x.
Interpretation	No filth should be visible under microscope. Light Filth must be absent in wheat flour
Reference	1. AOAC 17th edn, 2000, Official method 993.26 Light filth in Whole Wheat Flour 2. Glaze L. E and Bryce, J. R. 1994, Journal of AOAC INTERNATIONAL, 77, 1150–1152
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Moisture in Cereals and Cereal Products		
Method No.	FSSAI 03.005:2022	**Revision No. & Date**	0.0
Scope	A routine reference method for the determination of the moisture content of cereals and cereal products. It is applicable to the following products: wheat, durum wheat, rice (paddy, husked and milled rice), barley, millet rye, oats and rolled oats, triticale, sorghum and kaffir in the form of grains, milled grains, semolina or flour, pulses (whole and dehulled), macaroni products, solvent extracted and expellers pressed flours, cereal and legumes (pulses) flours, bran, cornflakes, corn flour couscous, tapioca sago and palm sago starch flour, textured soy protein products etc. The method is not applicable to malted foods.		
Caution	*Hot air oven*: Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature. Exercise extreme caution when opening and closing desiccators		
Principle	The sample is ground, after pre-conditioning, when required. A test portion is dried at a temperature of 130 ±3 °C to a constant mass. The loss in mass is expressed as a percentage.		
Equipment/Apparatus	1. Analytical balance (accuracy 0.001 g). 2. Grinding mill, having the following characteristics: • made of material which does not absorb moisture; • easy to clean and having as little dead space as possible; • enabling grinding to be carried out rapidly and uniformly, without appreciable development of heat and, as far as possible, without contact with the outside air; • adjustable so as to obtain particles pass through 1.0 mm IS sieve. Cold grinding mills can be used. 3. Moisture dishes – made of Aluminium or stainless steel ~7.5 mm wide and 2.5 mm deep with tight fitting lids. 4. Convection oven –thermostatically controlled to maintain temperature between 130 ±3 °C. 5. Desiccators containing desiccant (Silica gel/P_2O_5, $CaCl_2$).		
Sample preparation	*Macaroni products*: Select from lot to be analyzed enough strips or pieces to assure representative test sample, break these into small fragments with hands or in mill, and mix well. Grind 300–		

	500 g in mill until all material passes through No. 20 sieve. Keep ground test sample in sealed container to prevent moisture changes
Method of analysis	1. Weigh to the nearest 0.001 g, ~5.0 g of the laboratory sample in the-dish previously dried and weighed, together with its lid, to the nearest 0.001 g 2. Place the dish with its lid underneath in the oven for 2h. 3. The time should be reckoned from the moment the oven attains 130 °C after the dishes have been placed. 4. After 2h, cover dish while still in oven, transfer to desiccator. 5. Cool in the desiccator. 6. When the dish has cooled to room temperature (25 ±3 °C) (generally 30- 45 min after it has been placed in the desiccator), weigh it to the nearest 0.001 g. 7. The dish should be placed back in the oven till a constant weight is achieved.
Calculation with units of expression	The moisture content, expressed as a percentage by mass of the product, is given by the following equations $Moisture\ (\%) = \frac{W1-W2}{W1-W} \times 100.$ Where: W = Mass in g of the empty dish W1 = Mass in g of the dish with the test portion before drying W2 = Mass in g of the dish with the material after drying
Reference	1. IS 4333 (Part II): 2002 Methods of Analysis of food grains Part II Moisture 2. AOAC Official Method 926.06 Macaroni Products Preparation of Samples 3. AOAC Official Method 925.10 Solids (Total) and Loss on Drying (Moisture) in Flour Air Oven Method
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Moisture in Malted and Malt based foods: Vacuum Oven Method		
Method No.	FSSAI 03.006:2022	**Revision No. & Date**	0.0
Scope	A routine reference method for the determination of the moisture content of malt and malt-based foods		
Caution	*Vacuum oven*: are forbidden for use in unattended or non-working hours. Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature Exercise extreme caution when opening and closing desiccators		
Principle	The sample is ground, after pre-conditioning, when required. A test portion is dried at a temperature of 130 ±3 °C to a constant mass. The loss in mass is expressed as a percentage.		
Apparatus/Instruments	1. Vacuum oven 2. Analytical balance (Accuracy 0.001 g)		
Materials and Reagents	Flat Bottom Moisture Dish with Cover - stainless steel, nickel, aluminum or porcelain, of about 80 mm diameter and 25 mm height.		
Method of analysis	1. Weigh accurately about 5 g of the material into the dish previously dried and weighed. 2. Heat the dish containing the material after uncovering in the vacuum oven maintained at a temperature between 60 °C and 70 °C and at a pressure of not more than 7.500 mm of Hg for about 2 hours. 3. Cover and cool in a desiccator and weigh with the cover on. 4. Repeat the process of the drying, cooling and weighing at 30 minute intervals until the difference between the two consecutive weighing is less than 1 mg. 5. Record the lowest mass.		
Calculation with units of expression	The moisture content, expressed as a percentage by mass of the product, is given by the following equations $Moisture\ (\%) = \frac{W1-W2}{W1-W} \times 100.$ Where: W = Mass in g of the empty dish W1 = Mass in g of the dish with the test portion before drying W2 = Mass in g of the dish with the material after drying		
Reference	Indian Standard Specification for Malted Milk Foods IS : 1806 – 1975 Reaffirmed 2009		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Estimation of Uric Acid		
Method No.	FSSAI 03.007:2022	**Revision No. & Date**	0.0
Scope	This method describes the determination of uric acid in cereals and cereal products. The method is applicable toall food grains and their products.		
Caution	Concentrated Hydrochloric acid and Sulphuric acid: Handle with extreme care. Both these acidsare corrosive and can cause severe burns. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood **Sodium cyanide: Cyanide is a rapidly acting, poisonous chemical. Handle with extreme care and inform Head of laboratory when using** Prevent cyanide from being absorbed through the skin: • wear gloves when handling cyanide; • wear a protective apron and face shield whenever there is the slightest chance that you will be splashed; • do not rub your nose or eyes or pick your teeth when handling cyanide. If you have an itch - think before you scratch it! Do not bite your nails; • do not mop up perspiration with the sleeve of your overalls or with a cloth which is kept in the areas where cyanide is used or stored; • handle gloves, overalls and other protective equipment carefully and safely - wash immediately after use and store clean items well away from cyanide; and • wash your hands and face whenever you leave the areas where cyanide is used or stored.		
Principle	The method is based on the precipitation of proteins using sodium tungstate and sulphuric acid and treatment of protein free filtrate with Benedict's uric acid reagent and sodium cyanide. The resultant blue colour is measured colorimetrically/ spectrophotometrically against a similarly treated standard.		
Equipment/Apparatus	1. Analytical balance, capable of weighing to an accuracy of 0.001 g. 2. Photo-Electric Colorimeter or UV-Visible spectrophotometer 3. Volumetric flask - 50 mL capacity. 4. Burette 5. Glass Cuvettes or Nessler Tubes		

Materials and Reagents	1. Concentrated Sulphuric acid 2. Concentrated Hydrochloric acid 3. Sodium tungstate 4. Sodium cyanide 5. Standard Uric acid>99% pure 6. Phosphorus acid 7. Arsenic acid 8. Ammonia
Preparation of reagents	1. Sodium tungstate solution 10 % (w/v): Weigh 50 g of sodium tungstate and dissolve in 500 mL water. 2. Standard sulphuric acid solution (0.667 N) 3. Benedicts Uric acid reagent – Dissolve 100 g of pure Sodium tungstate in 600 mL water. Add 5 g of Arsenic acid (As_2O_3) followed by 25 mL of 85% phosphoric acid and 20 mL of concentrated HCl. Boil the mixture for 20 minutes, cool and make volume up to 1 L. 4. Sodium cyanide solution – 5% containing 2 mL of ammonia per L. This solution requires to be prepared fresh after about six weeks. 5. Standard Uric acid solution stock solution – Dissolve 9 g of Sodium dihydrogen phosphate in about 200 – 300 mL water. If the solution is not clear, filter and make upto 500 mL with hot water. Weigh 200 mg of pure uric acid in 1 L volumetric flask and add a few mLs of water to suspend the uric acid. Now add the solution made earlier and shake till the uric acid dissolves completely. Cool, add 1.4 mL of glacial acetic acid, dilute to mark and mix. Add 5 mL chloroform to prevent bacterial growth. 5 mL of stock solution contains 1 mg uric acid. 6. Working standard uric acid solution (0.02 mg/mL): Dilute 50 mL of stock solution containing 10 mg of uric acid with 400 mL distilled water in a 500 mL volumetric flask. Add 25 mL dilute HCl (1+9). Make the solution upto mark and mix. The working solution should be prepared from stock solution, which is not more than 10 days old.
Method of analysis	1. Weigh 50 g sample and grind it to a fine powder. 2. Take between 4-20 g powder expected to contain 1 to 5 mg uric acid and suspend in 200 mL water. 3. Allow the mixture to stand for 2 h and then mix in a Waring blender for 10 min and centrifuge at about 2000 rpm for 10 minutes. 4. To 100 mL of clear centrifugate add 10 mL sodium tungstate

	solution and mix. Then add 10 mL standard sulphuric acid solution to precipitate the proteins present in the extract. Mix and allow to stand for 5 minutes and filter. 5. Take an aliquot of the filtrate containing between 0.15-0.3 mg uric acid per 10 mL filtrate in the 50 mL volumetric flask and add 5 mL of sodium cyanide solution followed by 1 mL of Benedicts uric acid reagent. Shake gently and make upto mark with distilled water. 6. Determine the intensity of the colour in a spectrophotometer using 520 nm filter. Record the absorbance (A1) 7. Take 10 mL of standard uric acid solution containing 0.2 mg of uric acid in a 50 mL flask, add 5 mL of sodium cyanide followed by 1 mL of Benedicts uric acid reagent. Dilute to mark after 5 min and determine the intensity of colour at 520 nm (A2) 8. A parallel test using the same quantity of good uninfested sample as the sample under test should be run as a negative control.
Calculation with units of expression	$\text{Uric acid (mg/g)} = \frac{A1}{A2} \times \frac{12 \times 2 \times 0.2}{Mass\ of\ sample\ (g)}$
Reference	1. AOAC Method No. 970.24 can be used with applicable levels of more than or equal to 4 mg/100g 2. IS 4333 (Part 5) 1970 – Methods of Analysis for Food grains Part 5
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Ergot in Food Grains		
Method No.	FSSAI 03.008:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for the detection of Ergot in cereal grains		
Caution	Sulphuric acid: Handle with extreme care. Concentrated sulphuric acid is corrosive and can cause severe burns. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood. Ammonium hydroxide: Handle with extreme care. Avoid contact with eyes and skin. Eye contact may result in eye burns and temporary loss of sight. If inhaled, mild exposure can cause nose irritation. Handle only inside a fume hood. Diethyl ether*:* Extremely volatile and flammable. Handle with extreme care. Irritating to the eyes and the respiratory tract. Diethyl ether can de-fat the skin. Diethyl ether can form explosive peroxides under the influence of light and air. Keep away from heat and light. Handle only inside a fume hood. Store in a tightly sealed container in a cool room (preferably refrigerator) protected from light, moisture and air. Petroleum ether: Highly flammable liquid. Do not handle until all safety precautions have been read and understood. Use only in well ventilated areas. Avoid contact with all ignition sources, including hot surfaces. Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Keep container tightly closed in a dry and well-ventilated place. Containers, which are opened must be carefully resealed and kept upright to prevent leakage.		
Principle	Ergot alkaloids, a group of alkaloids also referred to as 'ergolines', contain essentially an indole nucleus. The indole nucleus of the ergot alkaloids reacts with *p*-dimethyl amino benzaldehyde in the presence of ferric chloride to give a deep blue color.		
Equipment/Apparatus	1. Grinding mill 2. Electric shaker 3. Analytical balance (Readability 0 0001 g) 4. Stoppered conical flask		
Materials/Reagents	1. Petroleum ether (40– 60 °C) 2. Diethyl ether 3. Dilute Ammonia 10% (v/v) 4. Tartaric acid		

	5. Concentrated sulphuric acid 6. *p*-Dimethyl amino benzaldehyde (PDAB) 7. Ferric chloride
Preparation of reagents	1. Ferric chloride (5% m/v): Dissolve 2.5 g of ferric chloride in 100mL of distilled water. 2. *p*-Dimethyl amino benzaldehyde (PDAB) reagent (Van Urk reagent)– Dissolve 0.125 g of PDAB in a cold mixture of 65 mL of concentrated Sulphuric acid and 35 mL of distilled water. Add 0.1 mL of 5% (w/v) ferric chloride solution and let it stand for 24 h before use. 3. Tartaric acid solution – 1% (w/v) (freshly prepared)
Method of Analysis	1. Grind about 50 g of sample in the grinding mill to a fine powder. 2. Take 10 g of powdered sample in a stoppered conical flask. 3. Add sufficient petroleum ether and shake for 30 min in the electric shaker. 4. Allow to settle and decant off the petroleum ether. 5. Dry the material in air. Then add 8 mL of dilute ammonia and sufficient quantity of diethyl ether. 6. Shake for 30 min. 7. Filter ether portion into a beaker and concentrate to a small volume. 8. Add 2 mL of tartaric acid solution to the beaker and shake thoroughly. Mix 1 mL of this tartaric acid – sample solution with 1 or 2 mL of pdimethyl benzaldehyde solution. 9. The appearance of blue colour indicates presence of Ergot.
Inference (Qualitative Analysis)	A deep blue color indicates the presence of Ergot.
Reference	IS 8184 :1976 Method of determination of Ergot in Food grains
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Hydrocyanic Acid in Beans: Alkaline titration		
Method No.	FSSAI 03.009:2022	**Revision No. & Date**	0.0
Scope	Applicable to beans, dry kidney shaped or flattened seeds of the leguminous varieties used as food, either whole or prepared as dal.		
Caution	Ammonium hydroxide: Handle with extreme care. Avoid contact with eyes and skin. Eye contact may result in eye burns and temporary loss of sight. If inhaled, mild exposure can cause nose irritation. Handle only inside a fume hood Acidification of cyanide solutions produces lethal, toxic hydrogen cyanide (HCN) gas. Carry out steam distillation within a ventilation hood. Wear hand gloves and eye protection at all times.		
Principle	The cyanogenic glucosides are hydrolysed and the liberated hydrocyanic acid (HCN) is steam distilled and titrated with silver nitrate in an ammoniacal medium in the presence of potassium iodide. Insoluble silver cyanoargentate (sometimes termed insoluble silver cyanide) is formed. In the Deniges modification, iodide ion (usually as KI, ca 0.01 M) is used as the indicator and aqueous ammonia is introduced to dissolve the silver cyanoargentate. The end point of the titration is characterized by the appearance of permanent turbidity due to precipitation of silver iodide.		
Apparatus/Instruments	1. Mechanical grinding mill 2. Analytical balance (Readability: 0.001 g) 3. Sieve with 1 mm aperture (No 20) 4. Volumetric flask 250 mL 5. Pipette 100 mL		
Materials and Reagents	1. Ammonium hydroxide solution 2. Potassium iodide 3. Silver nitrate 4. Sodium hydroxide		
Preparation of reagents	1. Ammonium hydroxide solution – Approx – 6 M prepared by diluting concentrated ammonia solution (0.9 g/mL) with an equal volume of water 2. Potassium iodide (KI) solution - Weigh 5.0 g of KI and		

	dissolved in 100 mL distilled water 3. Standard silver nitrate solution (0.02 M): 4. Sodium hydroxide solution(2.5%): 0.5 g in 20 mL water
Method of analysis	1. Grind a small quantity of the sample and reject it. 2. Then grind adequate quantity of the remaining sample to pass through a No 20 (1.0 mm) sieve. 3. Weigh 20 g of ground sample, transfer to 1 L distillation flask or 800 mL Kjeldahl flask, add 200 mL water and let stand for 2 h. Autolysis should be conducted with apparatus completely connected for distillation. Steam distill and collect 150- 160 mL distillate in sodium hydroxide solution (0.5 g in 20 mL water) and dilute to definite volume i.e 250 mL. 4. Take 100 mL, add 8 mL 6M ammonium hydroxide and 2 mL of KI solution. Titrate with 0.02 M silver nitrate using a micro-burette. 5. End point is faint but permanent turbidity and may be easily recognized, especially against black background.
Calculation and units of expression	1 mL of 0.02 M Silver Nitrate = 1.08 mg of HCN (Ag equivalent to 2CN)
Reference	1. AOAC Official Method 915.03 Hydrocyanic Acid in Beans Titrimetric Methods 2. IS 11535:1986/ISO 2164- 1975 Method of test for determination of glycosidic hydrocyanic acid in pulses
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Ash in Food grains and Food grain products		
Method No.	FSSAI 03.010:2022	**Revision No. & Date**	0.0
Scope	Ash refers to the inorganic residues remaining after either ignition or complete oxidation of organic matter. A routine reference method for the determination of the Total Ash content of cereals and cereal products. It is applicable to all food grins and products: wheat, durum wheat, rice (paddy, husked and milled rice), barley, millet rye, oats, triticale, sorghum and kaffir in the form of grains, milled grains, semolina or flour, biscuits and other bakery ware etc. The method is also applicable to edible starches and starch products such as tapioca Sago (Saboodana) and palm sago starch.		
Caution	Use safety thermally insulated gloves, tongs and protective eyewear while handling hot crucibles. During the analysis do not touch crucibles/dish with hands, but handle them with platinum-tipped tongs to avoid burns. Warm crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover slowly by sliding to one side to prevent a sudden inrush of air at the end of cooling period. Open and close desiccator slowly in order to avoid the danger of glass breakage.		
Principle	All organic matter is destroyed by incinerating the sample to a constant mass at high temperature of 550 ± 25 °C in a muffle furnace.		
Apparatus/Instruments	a. Dish, flat-bottomed, with surface area of at least 15 cm^2, made of Platinum, quartz, porcelain or any another material that remains unaffected by the conditions of the test. Crucibles/Dishes must be cleaned carefully. Never use abrasive products such as sand, hot concentrated nitric acid, free alkalis or aqua regia b. Muffle furnace, capable of being regulated at 550 ± 25 °C c. Desiccator, containing such as orange indicating silica gel d. Analytical balance, accurate up to 0.0001 g e. Special Muffle furnace tongs for crucible, stainless steel f. Thermal protection gloves, capable to resist temperature up to 550-600 °C		

	g. Bunsen burner h. Tripod stand, iron i. Wire gauze. j. Muffle furnace: 550±25 °C.
Method of analysis	1. Take fresh sample for the determination of Total ash, rather than left over after determination of moisture. 2. Weigh a previously clean and dried dish (W1). 3. Weigh accurately about 5 g of powdered sample into the dish. 4. Place with its lid underneath in the oven maintained at 130-133 °C for 2 h. 5. The time should be reckoned from the moment the oven attains 130 °C after the dishes have been placed. 6. Remove the dish after 2 h, cool in the desiccator and weigh (W2). 7. Ignite the dried material in the dish left after the determination of moisture with the flame of a burner till charred. 8. Transfer to a muffle furnace maintained at 550 ±25 °C and continue ignition till grey ash is obtained. 9. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing at 30 min intervals till the difference in weight in two consecutive weighing is less than 1 mg. 10. Note the lowest weight (W2). 11. If ash still contains black particles add 2-3 drops of pre-heated water at 60 °C. Break the ash and evaporate to dryness at 100-110 °C. Re-Ash at 550 °C. Until ash is white or slightly grey.
Calculation and units of expression	$\text{Total ash on dry basis (\% by weight)} = \frac{W2 - W}{W1 - W} \times 100$ Where, W = Mass in g of empty dish W1 = Mass in g of the dish with the dried material (moisture free) taken for test W2 = Mass in g of the dish with the ash Calculate the mean of two determinations and express the result to one decimal place

Reference	1. AACC (1995). "Ash – Basic method" in approved methods of the American Association of Cereal Chemists, 9th edition. 2. AOAC International (1995) « Ash of flour – direct method » in Official Methods of AOAC International, method 923.03 3. ISO 2171:1993 «Cereals and milled cereal products – Determination of total ash.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Acid Insoluble Ash		
Method No.	FSSAI 03.011:2022	**Revision No. & Date**	0.0
Scope	Acid Insoluble Ash refers to the ash remaining after dissolution of the total ash in dilute hydrochloric acid. This method is applicable to determination of acid insoluble ash of most of food grains including cereals and cereal products, pulses and their products, macaroni products, biscuits, bread and other bakery products.		
Caution	Concentrated hydrochloric acid: Handle with extreme care. Concentrated HCl is corrosive. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood. Muffle furnace: To avoid burns, do not touch the exterior or interior surfaces of this furnace during use or for a period of time after use. Wear the protective insulated glove. Use muffle furnace tongs for loading/unloading the furnace. Practice using the tongs before attempting to pick up a precious or extremely hot sample. Stand to one side when opening the door to avoid high temperature exposure.		
Principle	The total ash, is treated with dilute hydrochloric acid and filtered, incineration and weighing of the residue which is insoluble in acid.		
Apparatus/Instrument	1. Dish, flat-bottomed, with surface area of at least 15cm^2, made of platinum, quartz, porcelain or any another material that remains unaffected by the conditions of the test 2. Ashless filter paper (Whatman filter paper 42) 3. Water bath 4. Muffle furnace, capable of being regulated at 550 ± 10 °C 5. Electrical hotplate or surface heater 6. Fume hood or equivalent venting system 7. Desiccator, containing desiccant such as orange indicating silica gel 8. Analytical balance, accurate up to 0.0001 g 9. Tongs for crucible, stainless steel 10. Thermal protection gloves, capable to resist temperature up to 550-600 °C 11. Bunsen burner 12. Tripod stand, iron 13. Wire gauze		
Preparation of reagents	a. Dilute hydrochloric acid (~ 5.5 N): Into a 1000 mL volumetric flask, transfer with care about 600 mL water and 170 mL		

	concentrated hydrochloric acid (37%). Allow to cool to room temperature. Make up the mark with water. Mix well. ***Caution:*** Do not add water to acid. Always add acid to water b. Silver nitrate solution (10% m/v): Dissolve 10 g of silver nitrate in distilled water to a total volume of 100 mL.
Method of analysis	1. Add 15-25 mL of HCl solution to total ash of sample and boil for 10 min over a boiling water bath, covering the dish with watch glass to prevent spattering. 2. Filter the contents of the dish through the ashless filter paper. 3. Wash the dish and the filter paper with hot water until the washings are free from hydrochloric acid (about 6 to 8 times). Test for the absence of hydrochloric acid with silver nitrate solution. **Note:** Lack of turbidity when a portion of silver nitrate solution is added to the filtrate indicates absence of hydrochloric acid 4. Return the filter paper with the residue to the dish. 5. Evaporate it on water bath and ignite it in the Muffle furnace at 550 ± 10 °C for 1 h (or until the ash is carbon free). 6. When carbon-free ash is obtained, transfer the dish to desiccator, cool to 25±2 °C and weigh immediately. 7. Repeat the operations of igniting, cooling and weighing until the difference between successive weighing does not exceed 0.001 g (W2).
Calculation	Ash insoluble in dilute HCl on dry mass basis (A) $= \frac{W2 - W}{W1 - W} \times 100$ Where, W =Mass of empty dish in g W1= Mass of the dish with the dried ash portion taken for test W2= mass of dish and acid insoluble ash in g Calculate the mean of two determinations and express the result to one decimal place **Note:** Correct the acid insoluble ash weight for the blank of filter paper, if any Acid insoluble ash, % by mass (on dry basis) = (A x 100) 100-M Acid insoluble ash, % by mass (on dry basis) $= \frac{A \times 100}{100 - M}$

	Where: A = acid insoluble ash, percentage by mass and M = percentage of moisture in the bread
Reference	1. AOAC, Official Methods of Analysis. Association of Official Analytical Chemistry, Washington DC, 15th Ed. 1990. 2. Pearson, D. Egan, H. Kirl, R.S. and Sawyer, R. (1981) Pearson's Chemical Analysis of Foods Churchill Livingstone, Edinburg, 8th Ed.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Gluten content		
Method No.	FSSAI 03.012:2022	**Revision No. & Date**	0.0
Scope	The method described is applicable to the determination of gluten content of wheat flour (Atta), refined wheat flour (maida) and products containing wheat flour.		
Caution	Hot air oven: Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature.		
Principle	This test involves forming a dough and washing out the starch and water-soluble components (e.g. water-extractable pentosans, sugars, water-soluble proteins) from the dough. A wet and elastic substance made of water-insoluble proteins (gliadins and glutenins, about 85% total protein) remains after the washing procedure whose amount is an indication of gluten quantity. The total dry gluten (weight obtained after controlled drying of the wet gluten) is expressed as a percent.		
Apparatus/Instruments	1. Analytical balance: Accuracy (0.001g) 2. Porcelain cup 3. Spatula (for weighing and work dough) 4. Desiccator 5. Petri dishes 6. Convection oven: 133±1 °C 7. Tongs 8. Bolting silk cloth		
Materials and reagents	1. Potassium iodide 2. Iodine		
Preparation of reagents	2 % iodine in Potassium iodide solution: Dissolve 10 grams of potassium iodide crystals in 100 ml od distilled water. Swirl until crystals dissolve. Add 2 grams iodine and swirl until all iodine dissolves. Store in an dark brown bottle in the dark.		
Method of Analysis	1. Determine the moisture content of the flour. 2. Weigh 25 g flour into porcelain cup or mortar. 3. Add tap water to form a dough (50% water absorption is mostly used (0.5 g water/g of flour or 12.5 g water). 4. Knead (work) the dough by hand until a firm to medium consistency dough ball is obtained. Take care that all the material is taken into the dough. 5. Submerge the dough and soak with sufficient water in a beaker		

	for 60 min at 25±3 °C. 6. Knead dough gently (massage) while using wash water and replace with fresh water until starch and all soluble matter are removed. When much of the starch has been removed, the gluten ball will become darker and more elastic. 7. Remove the dough and place it in a piece of bolting silk cloth with an aperture of 0.16 mm (US Mesh 80) 8. Wash it with a gentle stream of water till water passing through the silk does not give a blue colour with a drop of iodine solution. 9. Spread the silk tight on a porcelain plate to facilitate scraping. Collect the residue to form a ball, squeeze in the palms to remove excess water. 10. Transfer gluten ball to a watch glass or petri dish and keep it in the oven at 133±1 °C for drying. 11. When partially dried, remove and cut into several pieces with a scissor and again keep in the oven to dry. 12. Cool in a desiccators and weigh. 13. Return it to the oven again for 30 min, cool and weigh to ensure constant weight.
Calculation and units of expression	Gluten (%) on dry weight basis $= \frac{Mass\ of\ dry\ gluten}{Mass\ of\ Sample} \times 100 \times \frac{100}{(100 - Moisture\ (\%)}$
Reference	1. IS 1155 :1968 Reaffirmed 2010 Indian Standard specification for Wheat atta(Second Revision) 2. AACC International. Approved Methods of Analysis, 11th Ed. Method 38-12.02. First approval November 8, 2000. Cereals & Grains Association, St. Paul, MN, U.S.A.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Gluten Content using Glutomatic equipment		
Method No.	FSSAI 03.013:2022	**Revision No. & Date**	0.0
Scope	This method specifies a method for the mechanical preparation of wet gluten and the subsequent determination of the dry Gluten content using the Glutomatic instrument. The method is applicable to whole wheat meals and all wheat flours.		
Caution	Follow the manufacturer's instructions while operating the instrument.		
Principle	Wet gluten is washed from whole-grain wheat meal or flour by an automatic gluten washing apparatus (Glutomatic) and centrifuged on an especially constructed sieve under standardized conditions. The total wet gluten is then dried under standardized conditions and weighed. The total dry gluten content is expressed as percentages of the sample.		
Apparatus/Instruments	Glutomatic system, which includes: 1. Glutomatic, with kneader, attachment for washing chambers, tubing and submersible filter for solvent container, and dough mixing and wash cycles for wheat meal. 2. Standard washing chambers with 88-µm polyester and 840-µm polyamide screens and screen holders. Metal chamber bottom for 840-µm screen is marked by a grooved ring. 3. Container for washing solvent, 10-liter or other size. 4. Dispenser, 0-5 mL or other range, adjustable in steps of 0.1 mL. 5. Centrifuge, operating at 6000 ± 5 rpm and equipped with gluten index cassettes. 6. Gluten dryer, with Teflon surfaces, drying at 150 °C for 4 min. 7. Laboratory mill, with 0.8-mm screen or mill that gives equivalent particle size with whole wheat. 8. Balance, accurate to 0.01 g.		
Chemicals/Reagents	The reagents used are to be of recognized analytical purity and quality. The water used is to be distilled. Sodium chloride		
Preparation of reagents	Sodium chloride solution20g/l (2%): Dissolve 200 g sodium chloride in water, dilute the solution by adding water to a total of 10 L. The solution should be prepared fresh daily. The temperature of wash solution should be 22 °C ± 2 °C.		
Sample preparation	Whole wheat meal is prepared by grinding wheat in a Laboratory equipped with an 0.8 mm sieve. For semolina/sooji grind to a fine powder and pass-through sieve.		

Method of Analysis	*Amount of test sample*: Ten g of the flour or meal to be tested is weighed accurately to 0.01 g and transferred without loss to the Glutomatic washing chamber. Make sure that the washing chamber is equipped with the fine 88 µm sieve and that the sieve is moistened. Shake the washing chamber gently to spread out the sample evenly. *Preparation of the dough*: Add 4.8 mL of the 2% sodium chloride solution. Hold the washing chamber at a slight angle and direct the water stream from the dispenser against the side wall, so that the water stream does not go directly though the sieve. Rock the washing chamber gently to spread the water evenly over the test sample. Note: In case of very weak gluten or very low gluten content the amount of added water may be diminished (down to 4.2 mL). At very high gluten content, the water added may be increased up to 5.2 mL. *Washing out gluten from wheat flour*: Place the washing chamber in the Glutomatic and start the test. Dough preparation in the washing chamber takes 20 seconds and the subsequent 5 min washing process is electronically controlled by the Glutomatic. *Removing gluten after the end of washing*: The Glutomatic gives a beep signal when 15 seconds remain of the washing sequence. When the Glutomatic stops, remove the washing chamber and take out the gluten carefully without stretching or tearing it. Ensure that no gluten remains on the mixing hook or in the washing chamber. Before the next test, clean the sieve carefully with water. *Centrifugation*: Push the gluten ball gently into the sieve cassettes. Do not divide the gluten in parts but put a gluten sample in each cassette. Start the centrifuge 30 seconds after the completion of the wash cycle. Centrifugation time is 60 seconds. After centrifugation, remove the sieve cassettes. Check that no gluten remains in the centrifuge. Using the stainless-steel spatula, carefully scrape off all gluten which has passed through the sieve. Weigh this portion to 0.01 g. Do not remove this portion from the balance. Using tweezers, pull out all gluten which has remained on the sieve and add this to the balance to achieve weight of total wet gluten. *Dry gluten content*

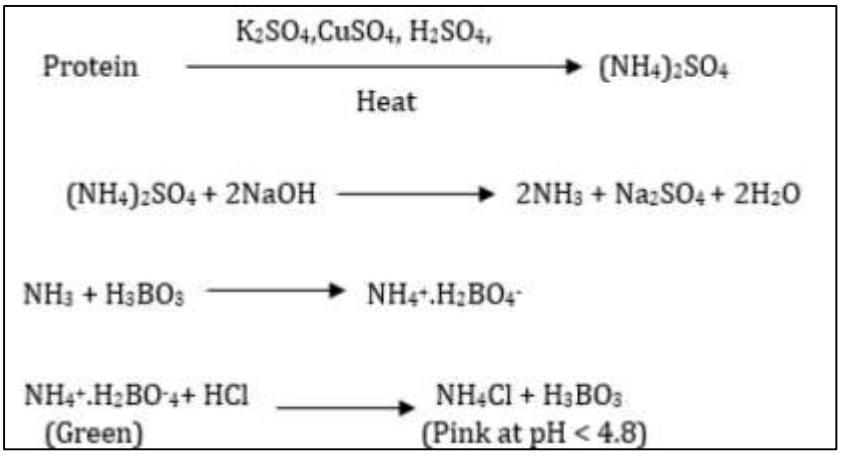
	1. Take total amount of wet gluten and place in center of lower heating surface of dryer. 2. Close dryer, and start drying at 150 °C for 4 min 3. With tweezers, carefully remove dry gluten from the dryer. Weigh dry gluten to nearest 0.01 g
Calculation and units of expression	Gluten (%) on dry weight basis $= \frac{Mass\ of\ dry\ gluten}{Mass\ of\ Sample} \times 100 \times \frac{100}{(100 - Moisture\ (\%)}$
Reference	1. AACC International Method 38-12.02 2. ICC STANDARD No. 155 Approved: 1994
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Alcoholic Acidity in Cereal and Grain Flours		
Method No.	FSSAI 03.014:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for all cereal and grain flours and first transformation products such semolina, grits etc.		
Caution	Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care		
Principle	Acid phosphates, amino acids and free fatty acids of flours, under certain conditions increase considerably due to the enzymatic hydrolysis of proteins and fats during storage. The amino acids and acid phosphates are soluble in strong alcohol. The free fatty acids are insoluble in water but are soluble in alcohol. For this reason, the acidity in flours is expressed as alcoholic acidity.		
Apparatus/Instrument	1. Conical flask 2. Analytical balance (Readability: 0.001g) 3. Pipette 4. Burette		
Materials/Reagents	1. Ethyl alcohol) 2. Sodium hydroxide 3. Phenolphthalein 4. Whatman filter paper No.1 or equivalent is to be used for filtration process		
Preparation of reagents	1. Neutral ethyl alcohol– 90% (v/v) 2. Standard sodium hydroxide solution – approx 0.05 N standardized using Potassium hydrogen phthalate 3. Phenolphthalein indicator – Dissolve 0.1 g in 100 mL of 60% Ethyl alcohol		
Method of analysis	1. Weigh 5 g of sample in a stoppered conical flask 2. Add 50 mL of neutral ethyl alcohol. 3. Stopper, swirl gently and allow to stand for 24 h with occasional swirling. 4. Filter the alcoholic extract through a dry filter paper. 5. Titrate the combined alcoholic extract with standardised sodium hydroxide solution to a pink end point using phenolphthalein as indicator. 6. Subtract titre value of blank alcohol.		

Calculation and expression of units	Alcoholic acidity (with 90 per cent alcohol) (% by mass on dry weight basis) $= \frac{24.52 \times A \times N}{M}$ Where: A= Titre value N=Normality of NaOH M= Mass of sample (dry weight basis)
Reference	1. IS 12711 :1989 Method of Determination of Alcoholic Acidity 2. IS: 1009 – 1979 Reaffirmed 2010 Specification for Maida p 10
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Acidity of Extracted Fat from Cereal Grains: Titrimetric Method		
Method No.	FSSAI 03.015:2022	**Revision No. & Date**	0.0
Scope	Acidity of extracted fat is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides. The method is applicable to all cereals, grains and oilseeds including Chia seeds.		
Caution	Toluene: Irritating to eyes, respiratory system and skin. Flammable and harmful. Avoid contact with skin and eyes. Keep container in a cool, well-ventilated area. Petroleum ether: Highly flammable liquid. Do not handle until all safety precautions have been read and understood. Use only in well ventilated areas. Avoid contact with all ignition sources, including hot surfaces Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Keep container tightly closed in a dry and well-ventilated place. Containers, which are opened must be carefully resealed and kept upright to prevent leakage. Potassium hydroxide is caustic. Contact with very high concentrations of may cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care		
Principle	The acid value is defined as the number of milligrams of Potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. The acid value is determined by directly titrating the extracted oil/fat in an alcoholic medium against standard potassium hydroxide solution.		
Apparatus/Instruments	1. Grain mill: Suitable for grinding small test samples. 2. Fat extraction device: Soxhlet or other suitable type. 3. Durable paper thimbles or Alundum RA-360 thimbles are suitable for extraction 4. Steam bath		
Materials and Reagents	1. Toluene AR Grade 2. Ethanol 3. Phenolphthalein 4. Potassium hydroxide 5. Petroleum ether 6. Potassium hydrogen phthalate		
Preparation of Reagents	1. Toluene–alcohol–phenolphthalein solution: 0.02%. To 1 L toluene add 1 L alcohol and 0.4 g phenolphthalein. 2. Alcohol–phenolphthalein solution: 0.04%. To 1 L alcohol add 0.4 g phenolphthalein. 3. Potassium hydroxide standard solution:0.0178M, carbonate-free. 1 mL = 1 mg KOH. Standardise with Potassium hydrogen phthalate		

Sample Preparation	1. Obtain representative test sample of ca 50 g grain/oilseeds by hand quartering or by use of mechanical sampling de vice. 2. Grind the test sample so that 90% will pass No. 40 sieve 3. If test sample is too moist to grind readily, dry at 100 °C just long enough to remove excess moisture.
Method of analysis	1. Extract 10 ± 0.01 g test portion with petroleum ether ca 16 h in extractor. 2. Start extraction as soon as possible after grinding and 3. never let ground test sample remain over-night. 4. Completely evaporate solvent from extract on steam bath. Dissolve residue in extraction flask with 50 mL toluene–alcohol–phenolphthalein solution. 5. Titrate with standard KOH solution to distinct pink, or in case of yellow solution to orange-pink. 6. If emulsion forms during titration dispel by adding second 50 mL portion toluene-alcohol-phenolphthalein solution. End point should match color of solution made by adding 2.5 mL 0.01% KMnO4 solution to 50 mL K2Cr2O7 solution of proper strength to match color of original solution being titrated (Add 0.5% K2Cr2O7 solution drop wise to 50 mL H2O until color matches. Then add 2.5 mL 0.01% KMnO4 solution.) 7. Make blank titration on 50 mL toluene-alcohol-phenolphthalein solution and subtract this value from titration value of test portion (V). Note If additional 50 mL portion toluene-alcohol-phenolphthalein solution was added, double blank titration.
Calculation with units of expression	$$Acidity\ of\ extracted\ fat\ (mg\ KOHper\ g) = \frac{56.1 \times V \times N}{W}$$ Where: V = Volume in mL of standard potassium hydroxide N = Normality of the potassium hydroxide solution W = Weight in gm of the sample
Reference	AOAC Official Method 939.05 Fat Acidity—Grains Titrimetric Method First Action 1939 Final Action
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Protein in all cereal and cereal based products		
Method No.	FSSAI 03.016:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for estimating the protein content of all wheat floursincluding protein rich wheat flour and protein rich refined wheat flour, maida, besan, , wheat semolina, malted and malt based products, solvent extracted flours, expeller pressed flours, flours from maize, ragi, pearl millet, sorghum, multigrain flours, soy protein ingredients, wheat protein products, degermed maize meal and Maize Grits, textured soybean products, tempeh, soybean beverages and related products, wheat bran etc. using the Kjeldahl method for nitrogen determination and a nitrogen to protein conversion factor of 6.25.		
Caution	Concentrated Sulphuric acid is highly corrosive and can cause severe burns. Handle with care Add hydrogen peroxide (30%w/w) after the addition of sulphuric acid to prevent explosions. This would prevent foaming caused by products with high fat content and foaming properties.		
Principle	The method is based on the principle that concentrated sulphuric acid in the presence of a catalyst helps in the digestion of food. All of the nitrogen is converted ammonium sulphate. By distillation in the presence of a base such as NaOH it is converted into ammonia. The ammonia is trapped in an acid (e.g. Boric acid), and titrated against 01N hydrochloric acid. The method involves the following reactions  		

	The nitrogen is converted into protein by multiplying with a conversion factor 6.25
Apparatus/Instruments	a. Kjeldhal flasks**:** Kjeldahl, hard, moderately thick, well-annealed glass, 500- or 800-mL capacity b. Distillation apparatus* c. Digestion apparatus. d. Conical or Erlenmeyer flask**:** 500 mL capacity, graduated at every 200 mL e. Burette**:** 50 mL capacity, graduated at least at every 0.1 mL or auto titrator may be used f. Boiling aids/Glass beads g. Measuring cylinders**:** 50-, 100- and 500-mL capacities, graduated h. Catalyst *Automated Kjeldhal digestion and distillation apparatus may be used. Follow the manufacturer's instructions
Materials/Reagents	a. Potassium sulfate (K_2SO_4): Nitrogen free or low in nitrogen content b. Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) c. Concentrated sulphuric acid: At least 95 - 98% (m/m), nitrogen free, ρ_{20} approximately = 1.84 g/mL d. Sodium hydroxide e. Methyl red f. Bromocresol green g. Boric acid h. Hydrochloric acid i. Standard Ammonium sulfate [$(NH_4)_2SO_4$]: Minimum assay 99.9% on dried material. Immediately before use dry the ammonium sulfate at 102 ± 2 °C for not less than 2 h. Cool to 25±2 °C in a desiccator. j. Tryptophan ($C_{11}H_{12}N_2O_2$) or Lysine hydrochloride ($C_6H_{15}ClN_2O_2$): Minimum assay 99%, do not dry these reagents in an oven before use. k. Sucrose with a nitrogen content of not more than 0.002% (m/m). Do not dry in an oven before use.
Preparation of reagents	a. Copper (II) sulfate solution: Dissolve 5.0 g of copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) in water and make up the final volume to 100 mL in a 100 mL volumetric flask b. Sodium hydroxide solution, 50%, (low in nitrogen): Dissolve 50 g NaOH pellets in water and finally make to 100 mL c. Indicator solution: Dissolve 0.1 g of methyl red in 95% (v/v) ethanol and dilute to 50 mL with ethanol. Dissolve 0.5 g of bromocresol green in 95% (v/v) ethanol and dilute to 250 mL

	with ethanol. Mix 1 part of methyl red solution with 5 parts of bromocresol green solution or combine all of both solutions. d. Boric acid solution (H_3BO_3): Dissolve 40 g of boric acid in hot water, allow the solution to cool and dilute to 1 L. Add 3 mL of methyl red - bromocresol indicator solution, mix and store the solution in borosilicate glass bottle. The solution will be light orange in colour. Protect the solution from light and sources of ammonia fume during storage. e. Standard hydrochloric acid solution: 0.1 ± 0.0005 N standardized with primary standard Sodium carbonate
Method of analysis	**Test portion and pre-treatment:** Add to the clean and dry Kjeldahl flask, 5 – 10 boiling aids, 15 g K_2SO_4, 1.0 mL of the copper sulfate solution, approximately 5 ± 0.1 g of prepared sample weighed to the nearest 0.1 mg, and add 25 mL of concentrated sulfuric acid. Use the 25 mL acid also to wash down any copper sulfate solution, K_2SO_4 or sample left on the neck of the flask. Gently mix the contents of the Kjeldahl flask. **Digestion:** Turn on the fume extraction system of the digestion apparatus prior to beginning the digestion. Heat the Kjeldahl flask and its contents on the digestion apparatus using a heater setting low enough such that charred digest does not foam up the neck of the Kjeldahl flask. Digest at this heat-setting for at least 20 min or until white fumes appear in the flask. Increase the heater setting to half way to the maximum setting as determined previously (See digestion apparatus) and continue the heating period for 15 min. At the end of 15 min period, increase the heat to maximum setting. After the digest clears (clear with light blue-green color), continue boiling for 1 h to 1.5 h at maximum setting. The total digestion time will be between 1.8 – 2.25 h. **Note:** At the end of digestion, the digest shall be clear and free of undigested material. Allow the acid digest to cool to 25±2 °C over a period of approximately 25 min. If the flasks are left on hot burners to cool, it will take longer to reach 25±2 °C . The cooled digest should be liquid or liquid with a few small crystals at the bottom of the flask at the end of 25 min cooling period. Do not leave the undiluted digest in the flask overnight. The undiluted digest may crystallize during this period and it will be very difficult to get that back into the solution to avoid this situation.

	Note: Excessive crystallization after 25 min is the result of undue acid loss during digestion and can result in low test values. Undue acid loss is caused by excessive fume aspiration or an excessively long digestion time caused by an incorrect maximum burner setting. After the digest is cooled to 25±2 °C , add 300 mL of water to 500 mL Kjeldahl flask or 400 mL of water when using 800 mL Kjeldahl flask. Use the water to wash down the neck of the flask too. Mix the contents thoroughly ensuring that any crystals which separate out are dissolved. Add 5 - 10 boiling aids. Allow the mixture to cool again to 25±2 °C prior to the distillation. Diluted digests may be stoppered and held for distillation at a later time. **Distillation:** Turn on the condenser water for the distillation apparatus. Add 75 mL of 50% (m/m) sodium hydroxide solution to the diluted digest by carefully pouring the solution down the inclined neck of the Kjeldahl flask, so as to form a clear layer at the bottom of the bulb of the flask. There should be a clean interface between the two solutions. Immediately after the addition of sodium hydroxide solution to the Kjeldahl flask, connect it to the distillation apparatus, the tip of whose condenser outlet tube is immersed in 50 mL of boric acid solution with indicator contained in a 500 mL Erlenmeyer flask. Vigorously swirl the Kjeldahl flask to mix its contents thoroughly until no separate layers of solution are visible in the flask any more. Set the flask down on the burner. Turn on the burner to a setting high enough to boil the mixture. Continue distillation until irregular boiling (bumping) starts and then immediately disconnect the Kjeldahl flask and turn off the burner. Turn off the condenser water. The distillation rate shall be such that approximately 150 mL distillate is collected when irregular boiling (bumping) starts and the volume of the contents of the conical flask will be approximately 200 mL. If the volume of distillate collected is less than 150 mL, then it is likely that less than 300 mL of water is added to dilute the digest. The efficiency of the condenser shall be such that the temperature of the contents of conical flask does not exceed 35 °C during distillation. **Titration:** Titrate the boric acid receiving solution with standard hydrochloric acid solution (0.1 N) to the first trace of pink colour.

	Take the burette reading to at least the nearest 0.05 mL. A lighted stir plate may aid visualization of the end point. **Blank test:** Simultaneously carry out a blank test by following the procedure as described above taking all the reagents and replacing the sample with 5 mL water and about 0.85 g of sucrose. **Note:** • The purpose of sucrose in a blank or a recovery standard is to act as organic material to consume an amount of sulfuric acid during digestion that is roughly equivalent to a test portion. If the amount of residual free sulfuric acid at the end of digestion is too low, the recovery of nitrogen by both recovery tests (See Section 19.1.1.3.4. i.e. Nitrogen recovery test) will be low. If the amount of residual acid present at the end of the digestion is sufficient to retain all the nitrogen, but the temperature and time conditions during digestion were not sufficient to release all the nitrogen from a sample, then the nitrogen recovery will be acceptable. • The amount of titrant used in the blank should always be greater than 0.00 mL. Blanks within the same laboratory should be consistent across time. If the blank is already pink before the beginning of titration, something is wrong. Usually, in such cases, the conical flasks are not clean or water from the air that may condense on the outside of the condenser apparatus has dripped down into the collection flask to cause the contamination. **Nitrogen recovery test** • The accuracy of the procedure should be checked regularly by means of following recovery tests, carried out in accordance with procedure as in the preceding steps • Check that no loss of nitrogen occurs by using a test portion of 0.12 g of ammonium sulfate along with 0.85 g of sucrose. Add all other reagents (except sample) as stated in Step A. Digest and distill under same conditions as for a sample. • The % of nitrogen recovered shall be between 99.0 and 100.0% for the given apparatus. In the case recoveries of nitrogen exceed 100%, ammonium sulfate is only useful to determine whether nitrogen loss has occurred or the normality of titrant is lower than the stated value. For recoveries less than 99%, the loss could be in the digestion or distillation step. It is

	possible to use a mixture of ammonium sulfate and small amount of sulfuric acid (the amount of residual remaining at the end of digestion) in a Kjeldahl flask. Dilute it with the normal value of water, add the normal amount of NaOH solution and distill. If the nitrogen recovery is still low by the same amount, the loss of nitrogen is in the distillation apparatus and not in that of the digestion. The probable cause might be leaky tubing in a traditional system or the tips of the condensers not submerged under the surface of boric acid solution early in the distillation. The apparatus should pass this test before going on to check recoveries by the procedure described below. • Check the efficiency of digestion procedure by using 0.16 g of lysine hydrochloride or 0.18 g of tryptophan along with 0.67 g of sucrose. Add all other reagents. Digest and distill under same conditions as for a sample. At least 98% of the nitrogen shall be recovered. If the recovery is lower than 98% after having a 99 - 100% recovery on ammonium sulfate, then the temperature or time of digestion is insufficient or there is undigested sample material (i.e., char) on the inside of the Kjeldahl flask. • The final evaluation of performance is best done by participation in a proficiency testing system, where within and between laboratories statistical parameters are computed based on analysis of samples. • Lower results in either of the recovery tests (or higher than 100% in case of ammonium sulfate) will indicate failures in the procedure and/or inaccurate concentration of the standard hydrochloric acid solution. ***Note*: Fully automated Kjeldahl Analyzer (digestion unit, distillation unit with integrated colorimetric titrator), can be used in place of the conventional system described**. Follow the manufacturer's instructions
Calculation and expression of units	Calculate the nitrogen content, expressed as a % by mass, by following formula $$W_n = \frac{1.4007 \text{ x } (V_s - V_B) \text{x } N}{W}$$ W_n=nitrogen content of sample, expressed as a % by mass;

	V_S=volume in mL of the standard hydrochloric acid used for sample; V_B=volume in mL of the standard hydrochloric acid used for blank test; N=Normality of the standard hydrochloric acid expressed to four decimal places; W= mass of test portion in g, expressed to nearest 0.1 mg. Express the nitrogen content to four decimal places. The crude protein content, expressed as a % by mass, is obtained by multiplying the nitrogen content by 6.25. Express the crude protein results to three decimal places. Protein = W_n x 6.25 Protein (dry weight basis) $=\frac{\text{Protein content} \times 100}{100 - \text{Moisture (\%)}}$
Reference	AOAC 979.09 (2005), Proteins in grains, Final action 1994. AOAC 976.05 (2005), Protein (crude) in animal feed and pet food, Final action 1977.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Crude Fiber		
Method No.	FSSAI 03.017:2022	**Revision No. & Date**	0.0
Scope	This method is for determination of crude fiber in in all food grain cereal and cereal products and is applicable to materials from which the fat can be and is extracted to obtain workable residue, including grains, meals, flours, feeds, and fibrous materials		
Caution	1. It is recommended to use fume-hoods. 2. Ethyl alcohol is flammable, handle with care. 3. Ensure neutralization of the acid/base used prior to disposal. 4. During digestion, heating shall be performed with care in order to avoid over-heating and too rapid boiling. 5. The foam formed in the vessel should never be allowed to exceed a height of 10 mm. 6. Sulfuric acid (H2S04) is a corrosive substance, destructive to the skin, eyes. Handle with care 7. Sodium hydroxide can cause severe skin burns and severe eye damage. Wear gloves and eye protection.		
Principle	Crude fiber is loss on ignition of dried residue remaining after digestion of sample with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH solutions under specific conditions. Separation of the residue by filtration followed by drying and ashing of the residue. The loss in weight resulting from ashing corresponds to the crude fiber content of the sample.		
Apparatus/Instrument	a. Soxhlet apparatus (optional) b. Digestion apparatus: With condenser to fit one-litre, digestion flask and hot plate adjustable to temperature that will bring 200 mL H_2O at 25 °C to rolling boil in 15 ± 2 min c. Digestion flask of such a size and shape that the solution will not be less than 1 inch (25 mm), nor more than 1.5 inch (38 mm) in depth. A one-litre Erlenmeyer flask with 45/50 ground joint is recommended. a. Ashing dishes: Silica, Vitreosil 70 x 16 mm; or porcelain, or equivalent b. Filtering device**:** Buchner Funnel. Alternatively, a filter cloth, of such character that no appreciable solid matter can pass through it during rapid filtration, may be used. Retention may be tested by running filtrate through a Gooch crucible. Butcher's linen, dress linen with ca. 45 threads to		

	an inch, or No. 40 filter cloth made by the National Filter Media Corporation, Hamden, connection 06514, or equivalent may be used. c. Desiccator with fresh and efficient desiccant (preferably, orange silica gel beads with moisture indicator). ***Note:*** Do not use silica with blue cobalt indicator, as it is not suitable for food applications**.** d. Analytical balance, accurate up to 0.0001 g e. Drying oven, capable of being controlled at 105± 1 °C f. Muffle furnace, capable of being regulated at 500 ± 25 °C
Materials/Reagents	a. Sulfuric acid, specific gravity 1.84 at 60 °F b. Sodium hydroxide pellets c. Ethyl alcohol, 95%, ACS grade d. Methylene chloride, anhydrous (dichloromethane), ACS grade e. Demineralized water f. Petroleum ether, initial boiling temperature, 35 °–38 °C; dry-flask end point, 52 °–60 °C; 95% distilling <54 °C, specific gravity at 60 °F, 0.630–0.660 g. Antifoam: Antifoam A compound diluted 1 + 4 with mineral spirits or petroleum ether, or H_2O-diluted antifoam B emulsion (1 + 4). Do not use antifoam spray. h. Blue litmus paper i. Bumping chips or granules: Broken Alundum crucibles or equivalent granules are satisfactory
Preparation of reagents	a. Sulphuric acid (H_2SO_4) solution, 0.255 N: Into a 1000 mL volumetric flask add about 200 mL of demineralized water then slowly introduce 12.5 g of conc. sulphuric acid and make up to the mark with demineralized water. Concentration must be checked by titration. If the concentration differs by more than ± 0.01 N from the nominal values adjust it within the range b. Sodium hydroxide (NaOH) solution, 0.312 N**:** Into a 1000 mL volumetric flask introduce 12.5 g of carbonate free sodium hydroxide pellets and make up to the mark with demineralized water. Concentration must be checked by titration c. Prepared ceramic fiber: Place 60 g ceramic fiber in blender, add 800 mL H_2O, and blend 1 min at low speed. Determine blank by treating 2 g (dry weight) of prepared ceramic fiber with acid and alkali as in determination. Correct crude fiber results for any blank, which should be negligible (2 mg).
Method of analysis	1. Weigh accurately about 2.5-3 g sample and transfer to an extraction apparatus (Soxhlet extractor) and extract with

	petroleum ether. Air dry the extracted sample and transfer to a dry 1 L conical flask. If percentage of fat in the product is high (>10%), then treat it with a mixture of acetone and petroleum benzene. Excess of fat, if not removed on initial defatting may affect the end result. 2. Add 200 mL of the H_2SO_4 solution connect the digestion flask to the condenser and place on a preheated hot plate or digestion rack adjusted so that the acid will boil in ca. 5 min. Continue boiling briskly for 30±1 min with frequent rotation of the flask to ensure thorough wetting and mixing of the sample. Material should not be allowed to remain on the sides of the flask out of contact with the solution. Add one drop diluted antifoam (Excess antifoam may give high results; use only if necessary, to control foaming.). Bumping chips or granules may also be added. Successive sample digestions should be started at ca. 3 min intervals to facilitate accurate timing. 3. After boiling 30 min, remove the flask and filter immediately through the Buchner funnel or through a filter cloth in a fluted funnel using a suction flask to speed filtration. Wash with boiling water until washings are no longer acid. Check alkalinity with litmus paper. 4. Transfer the sample and ceramic fiber quantitatively in digestion flask, washing the filter cloth or Buchner filter with 200 mL of NaOH solution. A wash bottle to deliver 200 mL is convenient. 5. Connect the flask to the reflux condenser, place on the preheated hot plate or heating mantle or digestion rack, bring to a boil in ca. 5 min, and boil exactly 28 min. Successive sample digestions should be started at ca. 3 min intervals to facilitate accurate timing. 6. Remove the flask and filter through fine linen (about 18 threads to a cm) held in a funnel and wash with boiling water until the washings are no longer acid to litmus (Crucible filter may be used in filtration steps as accidental tearing of linen may lead to safety concerns and also accuracy of results may be better with use of crucibles, Porosity 2 filter crucible, 50 mL volume- can be used).*Note: Filter aids can be added for better filtration and recovery of the analyte (filter aid Celite (R) 545).* 7. Bring to boil some quantity of sodium hydroxide solution. Wash the residue on the linen into the flask with 200 mL of boiling sodium hydroxide solution. 8. Immediately connect the flask to the reflux condenser and boil for exactly 30 minutes.

	9. Remove the flask and immediately filter through the filtering cloth. 10. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible prepared with a thin compact layer of ignited asbestos. 11. Wash the residue thoroughly first with hot water and then with about 15 mL of ethyl alcohol. 12. Dry the Gooch crucible and contents at 105±2 °C in an air oven until constant weight is achieved. 13. Cool and weigh. 14. Incinerate the contents of the Gooch crucible in a muffle furnace until all carbonaceous matter is burnt. 15. Cool the Gooch crucible containing ash in a desiccator and weigh (Dry the crucible with its residues in an oven at 130 °C for 2 h). • Limit of detection of approx 0.2g/100g crude fibre in the product. • Repeatability limit of 0.3 g/100 g when the crude fibre content is less than 10 g/100 g product and 3% of the average when the crude fibre content is equal to or greater than 10 g/100 g product. • Against use of asbestos, it is recommended to use filter aid Celite (R) 545, 22140 Fluka. ***Note*: Fully automated Crude Fibre Analyzer with filter bags or crucibles can be used in place of the conventional system described**. Follow the manufacturer's instructions.
Calculation and expression of units	The difference in weight of the crucible before and after ashing is reported as the crude fibre content of the test sample $$Crude\ Fibre\ (\%\ by\ mass) = \frac{W1 - W2}{W} \times 100$$ Where: W = Mass in g of the moisture free test material W1 = Mass in g of Gooch crucible and contents before ashing W2 = mass in g of Gooch crucible containing asbestos and ash Calculate crude fibre on dry wt. basis by giving correction for the moisture content.
Reference	AOAC, 2005, 962.09 Determination of crude fibre

Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Granularity in Maida (Refined Wheat flour): Sieving Method		
Method No.	FSSAI 03.018:2022	**Revision No. & Date**	0.0
Scope	Granularity is defined as that which passes through 212 µm (IS sieve 70 mesh). The method is applicable to refined wheat flour		
Principle	Granularity of a ground material, such as refined wheat flour, is the particle distribution of the material, which can be determined by a system of sieving. Data are reported as the weight of material remaining on a specified sieve or sieves after sieving for a standard time, expressed as a percentage of the original weight of the sample. For refined wheat flour, the weight of material passing through a 70-mesh sieve is used.		
Apparatus/Instrument	a. Analytical balance (Sensitivity 0.01 g) b. Sieve shaker c. Sieve (IS 70 mesh/212 µm)		
Method of analysis	1. Accurately weigh ca 50 ± 0.1 g of well-mixed, representative test 2. portion of refined flour. 3. Transfer test portion to the sieve with pan, and fixed in shaker 4. Shake 5 min. 5. Weigh, to the nearest 0.1 g, grits particles caught in pan. 6. Report % of fraction to 1 decimal place.		
Calculation and expression of units	$\text{Granularity (\%)} = \frac{Mass\ of\ flour\ collected\ in\ the\ pan}{Mass\ of\ sample} \times 100$		
Reference	AOAC Official Method 965.22Sorting Corn Grits Sieving Method First Action 1965 Final Action 1966		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Total Dietary Fibre in Flours: Rapid Integrated Enzymatic-Gravimetric–High Pressure Liquid Chromatography Method		
Method No.	FSSAI 03.019:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for the measurement of Total Dietary Fibre (TDF) by summing the quantity of higher molecular weight dietary fiber, which included insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) that precipitates in the presence of 78% aqueous ethanol (SDFP), with SDF that remains soluble in 78% aqueous ethanol (SDFS). It is applicable to all plant material, foods, and food ingredients.		
Caution	Some individuals are allergic to powdered pancreatic α-amylase (PAA) and/or amyloglucosidase (AMG) and/or AMG. In this instance, engage an analyst who is not allergic to prepare enzyme solutions. Do not add sodium azide to solutions of low pH. Acidification of sodium azide releases a poisonous gas. Handle sodium azide with caution only after reviewing SDS, using appropriate personal protective gear and laboratory hood).		
Principle	The procedure described is a "rapid" integrated TDF (RINTDF) method. Duplicate test portions of dried foods, fat-extracted if containing >10% fat, are incubated with pancreatic α-amylase (PAA) and amyloglucosidase (AMG) for 4 h at 37°C in sealed 250 mL bottles in a shaking water bath while mixing in orbital motion, or stirring with a magnetic stirrer, during which time nonresistant starch is solubilized and hydrolyzed to glucose and maltose by the combined action of the two enzymes. The reaction is terminated by pH adjustment followed by temporary heating. Protein in the sample is digested with protease. For the measurement of TDF, ethanol is added, and the IDF and SDFP are captured on a sintered glass crucible, washed with ethanol and acetone, dried, and weighed. One of the duplicate residues is analyzed for protein, the other for ash. SDFS in the filtrate is concentrated, deionized with resins, and quantitated by HPLC.		
Apparatus/Instruments	a. Grinding mill—Centrifugal, with 12 tooth rotor and 0.5 mm sieve, or similar device. Alternatively, cyclone mill can be used for small test laboratory samples provided they have sufficient air flow or other cooling to avoid overheating samples. b. Digestion bottles—250 mL Fisher brand soda glass, wide-mouth bottles with polyvinyl lined cap or equivalent c. Fritted crucible—Büchner, fritted disk, Pyrex 50 mL, pore size coarse, American Society for Testing and Materials 40–60 μm. Prepare each crucible as follows: ash overnight at 525 °C in muffle		

	furnace; cool furnace to 130 °C before removing crucibles to minimize breakage. Remove any residual Celite and ash material by using a vacuum. Soak in 2% micro cleaning solution, at room temperature for 1 h. Rinse crucibles with water and deionized water. For final rinse, use 15 mL acetone and air dry. Add approximately 1.0 g Celite to dried crucibles and dry at 130 °C to constant weight. Cool crucible in desiccators for approximately 1 h and record weight of crucible containing Celite. d. Filtering flask—Heavy-walled, 1 L with side arm. e. Rubber ring adaptors—For use to join crucibles with filtering flasks. Vacuum source—Vacuum pump or aspirator with regulator capable of regulating vacuum. f. Water bath(s)—Rotary motion, shaking, large-capacity (20–24 L) with covers; capable of maintaining temperature of 37 ± 1 and 60 ± 1°C. Ensure that shaking action/sample agitation in water bath is sufficient to maintain sample solids in suspension and that no residue buildup or rings of sample material form in the digestion bottle during the enzymatic digestions (i.e., at 150 rev/min;). If the water bath is used in linear motion (not preferred motion), then the bottles must be placed at an angle of 45° to the direction of movement to ensure continual suspension of the sample during the 4 h incubation period with PAA/AMG. Alternatively, mixing can be achieved with a submersible magnetic stirrer with a 30 × 7 mm stirrer bar, set at 170 rpm g. Analytical Balance (0.0001 g readability), h. Convection ovens—Two, mechanical convection, set at 103 ± 2 and 130 ± 3 °C. i. Timer j. Desiccator—Airtight, with SiO2 or equivalent desiccant. Desiccant dried biweekly overnight in 130 °C oven, or more frequently as needed. k. pH meter. l. Micropipettes and tips—50–200 µL and 5 mL capacity. m. Dispensers—(1)15 ± 0.5 mL for 78% EtOH, 95% EtOH and acetone. (2) 35 ± 0.2 mL buffer. n. Cylinder—Graduated, 100 and 500 mL. o. Magnetic stirrers and stirring bars. p. Rubber spatulas. q. Muffle furnace—525 ± 5 °C. r. Polypropylene tube—13 mL, 101 × 16.5 mm, flat base with screw cap s. Filters for water —Polyvinylidene fluoride, pore size 0.45 µm, 47 mm

	t. Filter apparatus—To hold 47 mm, 0.45 µm filter, to filter larger volumes of water. u. Syringes—10 mL, disposable plastic. v. Filters for disposable syringe- 0.45 mm (low protein binding Durapore PVDF), 25 mm or 13 mm or equivalent w. Syringes-Hamilton 100 µL, 710SNR syringe x. Microfuge centrifuge— Capable of 13,000 rpm. y. Rotary evaporator z. Thermometer—Capable of measuring to 100 °C aa. HPLC equipped with the following i. With oven to maintain a column temperature of 80 °C and a 50 µL injection loop. System must separate maltose from maltotriose. ii. HPLC columns—— Two LC columns connected in series. TSKgel® G2500PWXL, 7.8 mm id x 30 System must be capable of separating maltose from maltotriose with a run time of 60 min to ensure that all materials from the injection are cleared from the column prior to the next injection. iii. Cation and anion exchange guard column (containing deionizing/desalting cartridges)—Cation and anion exchange guard cartridges, H^+ and $CO2^{3-}$ forms respectively, with guard column holder to hold the two guard cartridges in series, cation cartridge preceding anion cartridge. iv. Guard column (or precolumn)—TSKgel PWXL guard column v. Detector—Refractive index (RI); maintained at 50 °C. vi. Data integrator or computer—For peak area measurement
Materials and Reagents	a. EtOH 95%, v/v. b. Acetone—Reagent grade. c. Stock PAA plus AMG powder—PAA (40 KU/g) plus AMG (17 KU/g) as a freeze-dried powder mixture. (Note: One Unit AMG activity is the amount of enzyme required to release one µmol d-glucose from soluble starch per minute at 40 °C and pH 4.5; one Unit PAA activity is the amount of enzyme required to release one µmol p-nitrophenyl from Ceralpha reagent per min at 40 °C and pH 6.9; AOAC 2002.01). PAA/AMG preparations should be essentially devoid of β-glucanase, β−xylanase and detectable levels of free d-glucose. d. Protease suspension (50 mg/mL, approximately 6 Tyrosine U/mg)—Stabilized suspension in 3.2 M ammonium sulphate. Swirl gently before use. Dispense using a positive displacement dispenser. Protease must be devoid of α-amylase and essentially

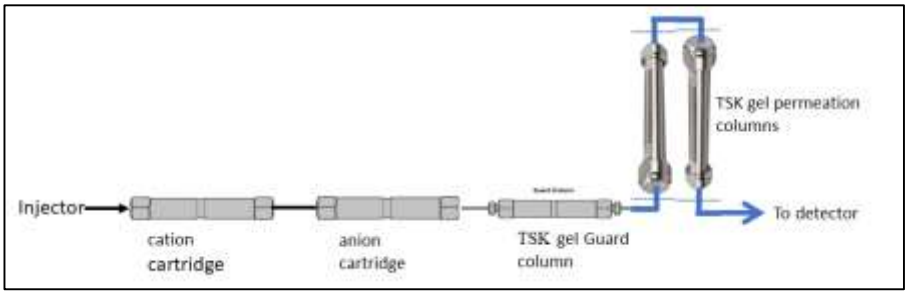
	devoid of β-glucanase and β-xylanase. Use as supplied. Stable for >4 years at 4 °C. e. Glycerol internal standard f. Diethyleneglycol g. Sodium azide h. LC retention time standard— Standard having the distribution of oligosaccharides (DP > 3) corn syrup solids (DE 25; Matsutani Chemical Industry Co., Ltd., Itami City, Hyogo, Japan; plus maltose in a ratio of 4:1 (w/w). i. d-Glucose j. Calcium chloride ($CaCl_2.2H_2O$) k. Sodium hydroxide l. MES [2-(N-morpholino) ethanesulfonic acid] m. Tris Base, n. Glacial acetic acid o. Cleaning solution—Micro (International Products Corp., Trenton, NJ). Make a 2% solution with deionized water. p. pH standards—Buffer solutions at pH 4.0, 7.0, and 10.0. q. Deionized water r. Celite—Acid-washed, pre-ashed. s. Amberlite FPA53 (OH^-) resin, ion exchange capacity 1.6 meq/mL (minimum) or equivalent t. Ambersep 200 (H^+) resin ion exchange capacity 1.6 meq/mL (minimum) or equivalent
Preparation of Reagents	a. EtOH 95%, v/v: It can be prepared by mixing 5 volumes of 2 propanol with 95 volumes of denatured ethanol formula SDA-3A (100 volumes of 95% EtOH combined with 5 volumes of methanol). b. EtOH (or IMS), 78%—Place 179 mL water into 1 L volumetric flask. Dilute to volume with 95% EtOH. c. PAA (4 KU/5 mL)/AMG (1.7 KU/5 mL)—Immediately before use, dissolve 1 g PAA/AMG powder in 50 mL sodium maleate buffer (50 mM, pH 6.0 plus 2 mM CaCl2) and stir for approximately 5 min. Store on ice during use. Use on the day of preparation d. Protease suspension (50 mg/mL, approximately 6 Tyrosine U/mg).— Use as supplied. Stable for >4 years at 4 °C. e. Glycerol internal standard—100 mg/mL containing sodium azide (0.02%, w/v). Stable for >4 years at 4 °C. f. Diethyleneglycol (100 mg/mL) in sodium azide (0.02%) is an alternative internal standard. This is less stable than the glycerol standard, so must be prepared on a weekly basis.

	g. LC retention time standard (maltodextrins)—Dissolve 1.25 g retention time standard in 30 mL of 0.02% sodium azide solution and transfer to a 50 mL volumetric flask. Pipette 5 mL glycerol internal standard (100 mg/mL). Bring to 50 mL with 0.02% sodium azide solution. Transfer solutions to 50 mL Duran bottle. Stable at 4 °C for >2 years and for > 4 year below -10 °C h. d-Glucose/glycerol LC standard—10 mg/mL of each containing sodium azide (0.02%, w/v). Stable for >4 years at 4 °C. i. (j) Sodium maleate buffer—50 mM, pH 6.0 plus 2 mM CaCl2 and 0.02% sodium azide. Dissolve 11.6 g maleic acid in 1600 mL deionized water and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g calcium chloride (CaCl2.2H2O) and adjust the volume to 2 L. Stable for approximately 2 weeks at 4°C. j. MES buffer—This can be used as an alternative to sodium maleate buffer; 50 mM, pH 6.0 plus 2 mM $CaCl_2$. Dissolve 19.5 g MES in 1600 mL deionized water, and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g calcium chloride ($CaCl_2.2H_2O$) and adjust the volume to 2 L. Solution is stable for approximately 2 weeks at 4 °C. k. Tris Base, 0.75 M.—Add 90.8 g Tris base to approximately 800 mL distilled water and dissolve. Adjust to pH 11.0. Adjust volume to 1 L. Stable for >1 year at room temperature. l. Acetic acid solution, 2 M.— Add 115 mL glacial acetic acid to a 1 L volumetric flask. Dilute to 1 L with distilled water. Stable for >1 year at room temperature. m. Sodium azide solution (0.02%, w/v)—Add 0.2 g sodium azide to 1 L deionized water and dissolve by stirring. Stable at room temperature for >1 year.
Sample Preparation	Collect and defat if >10% fat. For high-moisture samples, it may be desirable to freeze dry. Grind ca 50 g in a grinding mill, to pass a 0.5 mm sieve. Transfer all material to a wide-mouthed plastic jar and mix well by shaking and inversion. Store in the presence of a desiccant.
Method of analysis	**I. Enzymatic Digestion of Sample** Blanks—With each set of assays, run two blanks along with samples to measure any contribution from reagents to residue. Samples— (1) Weigh in duplicate 1.000 ± 0.005 g samples accurately into 250 mL polypropylene bottles. ***Step 1***: Wet the sample with 1.0 mL ethanol. Add 35 mL of 50 mM sodium maleate buffer or MES buffer, and a 7 × 30 mm stirrer bar to each bottle. Place bottles on a magnetic stirrer apparatus in a water bath set at 37 °C and stir the contents at 170 rpm for 10 min to equilibrate to 37 °C. Alternatively, transfer the bottles (without stirrer bar) to a shaking incubation bath, secure in place with the shaker frame springs,

	or a polypropylene holder and shake at 150 rpm in orbital motion for 10 min. ***Step 2****: Incubation with pancreatic α-amylase plus AMG*—Add 5.0 mL PAA/AMG solution, (PAA 4 KU/5 mL and AMG 1.7 KU/5 mL) to each bottle, cap the bottles, and incubate the reaction solutions at 37 °C with stirring at 170 rpm for exactly 4 h using a magnetic stirrer bar or a shaking water bath maintained at 37 °C at 150revolutions/min (orbital motion) for exactly 4 h. ***Step 3:*** *Adjustment of pH* to approximately 8.2 (pH 7.9–8.4). After 4 h, remove all sample bottles from the stirring or shaking water bath, and immediately add 3.0 mL of 0.75 M Tris base solutionto adjust pH to approximately 8.2 (7.9–8.4), at which pH AMG has no activity. ***Step 4:*** *Inactivation of PAA/AMG*: Immediately, slightly loosen the caps of the sample bottles, place the bottles in a boiling water bath (non-shaking; 95–100 °C), and incubate for 20 min with occasional agitation (by hand). This inactivates both PAA and AMG. With a thermometer, ensure that the final temperature of the bottle contents is >90 °C. Checking just one bottle is adequate. (At the same time, if only one shaker bath is available, increase the temperature of the shaking incubation bath to 60 °C in readiness for the protease incubation step). **Step 5:** *Cooling and protease treatment*—Remove all sample bottles from the hot water bath and cool to approx. 60 °C. Add 0.1 mL protease suspension, with a positive displacement dispenser (solution is thick) and incubate at 60 °C for 30 min. Step 6: pH adjustment—Add 4.0 mL of 2 M acetic acid, to each bottle and mix. This gives a final pH of approximately 4.3. Step 7: Add internal standard—To each sample, add 1 mL of 100 mg/mL glycerol (or diethyleneglycol) internal standard solution. **I. Determination of IDF + SDFP** ***Step 1***: Precipitation of SDFP and recovery of IDF + SDFP. To each sample, add 207 mL (measured at room temperature) of 95% (v/v) EtOH and mix thoroughly. Allow the precipitate to form at room temperature for 60 min (overnight precipitation is acceptable). ***Step 2****: Filtration setup*—Tare crucible containing Celite to nearest 0.1 mg. Wet and redistribute the bed of Celite in the crucible, using 15 mL of 78% (v/v) EtOH from wash bottle. Apply suction to crucible to draw Celite onto fritted glass as an even mat. Discard these washings. ***Step 3****: Filtration*—Using vacuum, filter precipitated enzyme digest, through crucible. Using a wash bottle with 78%, v/v EtOH, quantitatively transfer all remaining particles to crucible and wash the residue successively with two 15 mL portions of 78%, v/v EtOH Retain filtrate and washings for determination of SDFS. ***Step 4:*** *Wash*.—Transfer the crucible to a “waste” Buchner flask and,

	using a vacuum, wash residue successively with two 15 mL portions of 95% (v/v) EtOH and then acetone. Discard these washings. Draw air through the crucibles for at least 2 min to ensure all acetone is removed before drying crucibles in an oven. ***Step 5:*** *Dry crucibles*—Loosely cover the crucibles with aluminium foil to prevent sample loss, and then dry the crucibles containing residue overnight in a 103 °C oven. ***Step 6****: Cool crucible*—Cool crucible in desiccators for approximately 1 h. Weigh crucible containing dietary fiber residue and Celite to nearest 0.1 mg. **Step 7:** Calculated IDF + SDFP (by gravimetry) as shown below.To obtain residue weight, subtract tare weight, i.e., weight of dried crucible and Celite. III. **Protein and ash determination** 1. The residue from one crucible is analyzed for protein, and the second residue of the duplicate is analyzed for ash. 2. Perform protein analysis on residue using Kjeldahl method. Use 6.25 factor for all cases to calculate g of protein. 3. For ash analysis, incinerate the second residue for 5 h at 525 °C. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash. **IV. Checking the adequacy of deionsing capacity of resins** **Note:** Proper deionization of the filtrate (Step 3 of III) is an essential part of obtaining quality chromatographic data on SDFS. To ensure that the resins being used are of adequate deionizing capacity: 1. Add 0.1 mL protease suspension, to 40 mL either maleate buffer, or MES buffer, along with 3.0 mL of 0.75 M Tris base solution, 4.0 mL of 2 M acetic acid, 1 mL glycerol internal standard (100 mg/mL), and 1 mL d-glucose solution (100 mg/mL). 2. Concentrate this solution to dryness on a rotary evaporator and redissolve the residue in 32 mL deionized water. 3. To 5 mL of this solution in a 13 mL polypropylene tube add 1.5 g Amberlite FPA53 (OH^-) resin, and 1.5 g Ambersep 200 (H^+) resin, and swirl the contents regularly over 5 min. 4. Allow the resin to settle and remove the supernatant (1.5–2.0 mL) with a syringe, and filter through a polyvinylidene fluoride filter, pore size 0.45 µm. 5. Inject an aliquot (50 µL) of this solution onto the HPLC connected with TSKgel G2500PWXL columns. No salt peaks

	should be seen on the chromatogram. **V. Determination of SDFS** ***Step 1:*** *Filtrate recovery, deionization* 1. Use the filtrate (Step 3 of III) from one of the sample duplicates to use in case of spills or if duplicate SDFS data are desired. 2. Transfer the filtrate from the second sample replicate, into a 500 mL measuring cylinder. 3. Adjust the volume to 300 mL with 78% (v/v) aqueous ethanol, transfer to a 1 L beaker, and mix thoroughly. 4. Transfer approximately 75 mL (approximately 25%) of this solution to a 500 mL evaporator flask and concentrate with a rotary evaporator to dryness at 50 °C. (Note: it is not essential to quantitatively transfer all solution because SDFS is determined by the ratio of these peaks on HPLC to that of glycerol internal standard). ***Step 2****: Deionization of sample* Dissolve the residue in the evaporator flask in 8 mL deionized water and transfer 5 mL of this solution to a 13 mL polypropylene tube, containing 1.5 g Amberlite FPA53 (OH^-) resin and 1.5 g Ambersep 200 (H^+). Cap the container and invert the contents regularly over 5 min. Note Alternatively, if the ammonium sulphate suspension of PAA/AMG is used for starch digestion then use 2 g Amberlite FPA53 (OH−) resin and 2 g Ambersep 200 (H+) to ensure effective removal of most of the ions in the sample. ***Step 3*** *:Prepare samples for LC analysis.* 1. Remove a sample (approximately 1.5–2.0 mL) of the supernatant solution from the resin slurry with a syringe 2. Filter through a polyvinylidene fluoride filter, pore size 0.45 μm, 3. Use this solution as the sample extract for HPLC analysis. ***Step 4:*** *HPLC conditions* 1. Columns: Two TOSOH TSK gel permeation columns in series with guard column at 80 °C pre ceded by two deionising pre-

	columns as shown in figure below  2. Cation column to precede anion cartridge 3. Mobile phase: Microfiltered distilled water. 4. Flow rate: 0.5 mL/min; 60 min per run. 5. Column Oven Temperature: 80 °C 6. Detector RI at 50 °C ***Step 4 Determine the response factor for d-glucose.*** Note: Because d-glucose provides an LC RI response equivalent to the response factor for the nondigestible oligosaccharides that make up SDFS, d-glucose is used to calibrate the LC and the response factor is used for determining the mass of SDFS. 1. Use a 100 µL LC syringe to fill a 50 µL injection loop with the D-glucose/glycerol internal standard solution. Inject in duplicate. 2. Calculate the response factor. ***Step 5** : Calibrate the area of chromatogram to be measured for SDFS* 1. Use a 100 µL LC syringe to fill the 50 µL injection loop with retention time standard. Inject in duplicate. 2. Determine demarcation point between DP 2 and DP 3 oligosaccharides (disaccharide maltose versus higher oligosaccharides) ***Step 6**: Determine peak area of SDFS (PA_{SDFS}) and internal standard (PA_{IS}) in chromatograms of sample extracts.* 1. Inject sample extracts on LC. 2. Record area of all peaks of DP greater than the DP2/DP3 demarcation point as PA_{SDFS}. 3. Record the peak area of internal standard as PA_{IS}.
Calculation with units of expression	Calculation of Total Dietary Fiber as sum of 1) HMWDF (IDF + SDFP) and 2) SDFS: **(a) HMWDF (IDF + SDFP) (by gravimetry).** (1) Blank determination (B, mg)

$$B\ (mg) = \frac{BR1 + BR2}{2} - PA - PB$$

where BR1 and BR2 = residue mass (mg) for duplicate blank determinations respectively; PB and PA = mass in mg of protein and ash respectively, determined on first and second blank residues.

(2) [IDF + SDFP] determination.

$$[\text{IDF} + \text{SDFP}]\frac{\text{mg}}{100\text{g}} = \frac{= [\frac{\text{R1} + \text{R2}}{2}] - \text{PB} - \text{PA} - \text{B}}{(\text{M1} + \text{M2})/2} \times 100$$

$$[\text{IDF} + \text{SDFP}]\ \text{g}/100\ \text{g} = \frac{[\text{IDF} + \text{SDFP}]\ \text{mg}/100\ \text{g}}{1000}$$

where R1 = residue mass 1 from M1 in mg; R2 = residue mass 2 from M2 in mg; M1 = test portion mass 1 in g; M2 = test portion mass 2 in g; PA = ash mass from R1 in mg; PB = protein mass from R2 in mg; B = determined value for the Blank in mg.

(b) SDFS (by HPLC).

(*1) Determination of D-glucose response factor*.

Obtain the values for the peak areas of D-glucose and internal standard (glycerol) from duplicate chromatograms. The ratio of peak area of D-glucose/peak area of glycerol to the ratio of the mass of D-glucose/mass of glycerol is the "response factor." The average response factor for D-glucose is approximately 0.82 verses glycerol.

$$\text{Response factor (Rf)}\ = \frac{PA_{IS}}{PA_{Glu}} \times \frac{Wt_{IS}}{Wt_{Glu}}$$

where PA_{Glu} = peak area D-glucose; PA_{IS} = peak area internal standard (glycerol); Wt_{Glu} = mass of D-glucose in 1 mL of D-glucose/ glycerol standard (10 mg); Wt_{IS} = mass of internal standard (glycerol) in 1 mL of D-glucose/glycerol standard (10 mg).

(2) Determination of SDFS.

$$\text{SDFS}\ \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Rf x}\ Wt_{IS}\ \text{x}\ PA_{SDFS}}{\text{PA}_{IS}} \times \frac{100}{M}$$

$$\text{SDFS}\ \left(\frac{\text{g}}{100\text{g}}\right) = \frac{\text{SDFS}\ \left(\frac{\text{mg}}{100\text{g}}\right)}{1000}$$

where Rf = the response factor; Wt_{IS} = mg of internal standard contained in 1 mL of glycerol internal standard solution (100 mg/mL, i.e. 100 mg) pipetted into sample before filtration; PA_{SDFS} = the peak

	area of the SDFS fraction; PA = the peak area of the glycerol internal standard; M = the test portion mass (M1 or M2) in grams of the sample whose filtrate concentrated and analyzed by LC. *(c) Total Dietary Fiber*. Total dietary fiber (g/100g) = [IDF + SDFP] g/100g) + SDFS (g/100g)
Reference	AOAC Method 2017.16 (RINTDF assay procedure). Total Dietary Fiber in Foods (Codex Definition) by a Rapid Enzymatic-Gravimetric Method and Liquid Chromatography. McCleary, B. V. (2019). Total dietary fiber (CODEX definition) in foods and food ingredients by a rapid enzymatic-gravimetric method and liquid chromatography: collaborative study, First Action 2017.16. J. AOAC Int., 102, 196-207 McCleary, B. V., DeVries, J. W., Rader, J. I., Cohen, G., Prosky, L, Mugford, D. C. & Okuma, K. (2012). Determination of insoluble, soluble, and total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: collaborative study. J. AOAC International, 95, 824-844.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Kesari Dal Powder (*Lathyrus sativus*) in Besan flour.		
Method No.	FSSAI 03.020:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for detecting the adulteration of besan (Chickpea flour) with Kesari dhal flour		
Caution	Suitable precautions while drying the chromatograph shall be taken to avoid ingestion of hazardous vapours.		
Principle	The presence of Kesari dal powder is detected on the basis of the presence of an unusual nonprotein amino acid namely Beta-N-oxalyl-L amino alanine (BOAA), which is not present in the seeds of other legumes. Free amino acids are separated by paper chromatography and detected with ninhydrin spray.		
Apparatus/Instrument	a. Analytical balance (Accuracy 0.001g) b. Steam bath/water bath c. Hot Air Oven d. Chromatographic paper Whatman No. 1 or equivalent e. Capillary tubes or 10 µL syringe		
Materials/Reagents	a. Pyridine (Chromatography grade) b. Glacial acetic acid c. Ethyl alcohol: 70%(v/v) d. Isopropanol solution: 10% (v/v) e. Liquified distilled Phenol: water solution - (4: 1) f. Ninhydrin solution – 0.1% in acetone or ethanol g. Standard β-N-oxalyl-2,3-diaminopropionic acid (BOAA)		
Preparation of reagents	a. Liquefied distilled(glass) phenol: distilled water 4:1 b. Ninhydrin spray: Dissolve 100 mg of ninhydrin in acetone		
Sample preparation	1. Weigh approximately 1 g powdered sample 2. Extract it with 100 mL ethyl alcohol (70%) by keeping overnight, with shaking. 3. Filter the extract and evaporate to dryness on a steam/water bath. 4. Extract the residue with 10 mL of 10% isopropanol solution, filter and use this solution for chromatography		
Method of analysis	1. Spot 20 µL of the extract using a graduated capillary tube or syringe at a distance of 1 cm from the bottom of the		

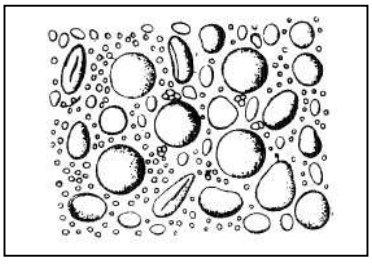
	chromatographic filter paper. 2. Also spot as standard BOAA and extracts from Kesari dhal and pure Besan(Chickpea) powder 3. Develop in a solvent chamber saturated with phenol–water solution overnight. 4. Remove from the chamber, dry chromatogram in a current of air at room temperature for 4-5 hour in an oven at 80 °C for 1 h and spray with ninhydrin solution. 5. Dry the chromatogram in the oven at 100 °C for 5 minutes. 6. The appearance of bluish – purple spot at about R_f value 0.1 shows presence of BOAA which is present only in *Lathyrus sativus*. 5. Other amino acids extracted simultaneously also give similar colour but at different R_f values. 6. Always run standard BOAA and a known sample of Kesari dal powder simultaneously and compare the R_f.
Interpretation	The appearance of a bluish spot with Rf similar to standard or Kesari dhal extract indicates the presence of Kesari dhal powder in Besan flour.
Reference	ISI Handbook of Food Analysis (Part IV) – 1984 Page 121
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Kesari Dal Powder (*Lathyrus sativus)* in Besan by Capillary Electrophoresis		
Method No.	FSSAI 03.021:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to all legume and grain flours		
Principle	Seed flour is extracted with ethanol-water (6:4) and the extracted free amino acids separated by capillary zone electrophoresis(CZE) with a 50 m uncoated capillary in Na_2HP04 buffer at pH 7.8 with direct detection at 195 nm. A linear response was recorded in the concentration range 0.015-1.8 mM. This corresponds to a detection limit of 0.1 g /kg		
Apparatus/Instrument	1. A capillary electrophoresis (CE) instrument 2. equipped with a diode array detector set at 195 nm via software. 3. The capillary (HP 4. G1600-60211 or equivalent) of uncoated fused silica had the dimensions 48.5 cm x 50 µm and an effective length of 40 cm. 5. Centrifuge 6. Analytical balance (Readability 0.01g)		
Materials/Reagents	1. 3-(N-Oxalyl)-~-2,3-diaminopropanoic acid (β-ODAP), 2. Hippuric acid, 3. 2,3-diaminopropionic acid (DAP), 4. Disodium hydrogen monophosphate (Na_2HPO_4) 5. Monosodium dihydrogen phosphate (NaH_2PO_4 6. Dimethyl sulfoxide (DMSO) 7. L-Asparagine (L-Asn) and other protein amino acids		
Sample preparation	a. Seed flour (0.5 g) was extracted in 2 volumes of 10 mL of ethanol-water (6:4) by tumbling in capped plastic vials (14 mm i.d. x 10 cm) for 45 min each time. b. Hippuric acid (12.84 mM), which was pre-dissolved in dimethyl sulfoxide (DMSO, 3% v/v of final volume) was used as internal standard, and 200 µL of solution was added to the flour prior to the first extraction. c. The tubes were vortexed before tumbling. d. The extracts were centrifuged at 1400g for 10 min. e. The pooled extracts were filtered prior to CZE analysis using syringe filters (0.45 µm)		
Method of analysis	a. The capillary was conditioned prior to each run by flushing it with 0.1 M NaOH for 2 min and with the electrolyte for 3 min.		

	b. The analyses were performed at a constant voltage of 25 kV at 40 °C in an electrolyte of 20 mM Na_2HP04 buffer at pH 7.8. c. The electrolyte was replenished every third run. d. Filtered seed extracts were injected for 4-12 s at 25 mbar depending on the concentration of the extract e. Detection was carried out at 195 nm
Interpretation	CZE analyses of fresh standard solutions of β-ODAP showed one major peak with a migration time of 1.18 relative to that of the internal standard and a minor peak moving slightly more quickly (Mt_{rel}= 1.13). The area of the latter accounted for 2.6% of the combined area of the two.
Reference	Arentoft and Greirson (1995) Analysis of 3-(N-Oxalyl)-L-2,3-diaminopropanoic Acid and Its alpha-Isomer in Grass Pea (*Lathyrus sativus*) by Capillary Zone Electrophoresis J. Agric. Food Chem., 43, 942-945
Approved by	Scientific Panel on Methods of Sampling and Analysis

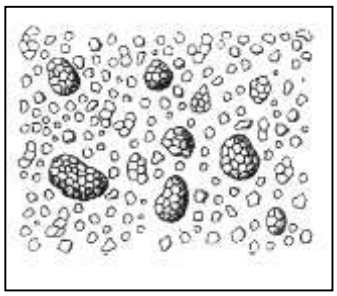
	Determination of Talc in Rice and Pulses		
Method No.	FSSAI 03.022:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to Rice and pulses		
Caution	Ammonia: Handle with extreme care. Avoid contact with eyes and skin. Eye contact may result in eye burns and temporary loss of sight. If inhaled, mild exposure can cause nose irritation. Handle only inside a fume hood. Concentrated HCl: Handle with extreme care. Avoid contact with eyes and skin. Eye contact may result in eye burns and temporary loss of sight. If inhaled, mild exposure can cause nose irritation. Handle only inside a fume hood		
Principle	The talc is floated off, filtered, digested, ignited and weighed**.**		
Apparatus/Instruments	Analytical balance		
Materials/Reagents	a. 10 % Ammonia solution b. 3 % Hydrogen peroxide c. Chromium trioxide d. Concentrated HCl		
Preparation of reagents	Hydrochloric- chromic acid mixture – Carefully dissolve 10 g of Chromium Trioxide in 100 mL of water and add to 900 mL of concentrated hydrochloric acid		
Method of analysis	a. Shake 20 g of sample with the ammonia (10 %) and hydrogen peroxide (3%) solutions. b. Heat to about 60 °C so that the gas formed causes the particles of talc to come away from the surface. c. Decant off the liquid containing talc d. Wash the grains several times with water and add these washings to the decanted liquid. e. Heat the liquor with the Hydrochloric- Chromic acid mixture to oxidize suspended meal, f. Filter off the talc, g. Wash, ignite and weigh		
Calculation/Interpretation	In unpolished rice, the talc residue does not normally exceed 0.025%. Talc (%) $=\frac{W1}{W2} \times 100$ Where: W1 = Mass of Talc W2=Mass of sample		
Reference	Manuals of food quality control 8. Food analysis: quality, adulteration and tests of identity 14/ 8, page 200, Reprinted 1997		

Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Microscopic Structure of Cereal Starches		
Method No.	FSSAI 03.023:2022	**Revision No. & Date**	0.0
Scope	This visual examination method is applicable to cereal starches		
Principle	Starch grains vary in size, shape, and form and can be used to identify the cereals they originate. Starch grains are visualized and then viewed under a microscope		
Apparatus	a. Microscope – with an eye piece micrometer calibrated with a slide micrometer and having a magnification of 300 – 500 b. Microscopic slides c. Cover slips - circular or square		
Method of analysis	1. Take a small quantity of the sample (1 g or less) in a test tube or beaker and add about 50 mL water. 2. Stir the contents with the help of glass rod to break up granules and lumps if any. 3. Let it stand for a few minutes. 4. Place a drop of the suspension on a microscopic glass slide and press a cover slip on the drop of suspension taking care that no air is trapped between the slide and cover slip. 5. Remove excess liquid on the slide with a piece of blotting paper. 6. Examine the slide under the microscope		
Interpretation	**Wheat starch** The small grains vary from 2 µm to 8 µm in diameter averaging about 6 -7 µm They are rounded or oval in outline, seldom polygonal or pointed. The large grains in surface view appear sometime rounded, sometime slightly irregular or oval but when touching the cover slip with the needle they are made to present their edges to the observer, they are seen to be flattened or lenticular in shape. They seldom exhibit concentric striate or evident hilum. The photomicrograph is shown below. 		

Rice Starch

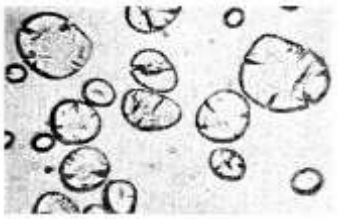
It consists of both simple and compound grains. The simple grains are tolerably uniform in size and shape and range from 4 to 6 µm sometime reaching 8 µm and are generally angular. The compound grains are ovoid or rounded in shape but vary very much in size according to the number of constituent grains that they contain. The starch closely resembles oat starch. When treated with water the compound grains are readily dissociated in their constituent grains and normally the former are seldom found in the rice starch of commerce. The photo micrograph is given below.



Tapioca Starch

Tapioca starch is obtained from Cassava (*Manihot utilissima*) and other species of *Manihot* by heating and stirring the moist starch until it agglutinates into a little irregular and rugged mass which is known commercially as tapioca.

The grains of Cassava are originally compound, consisting of two, three or four component grains and is occasionally found intact. Most of them however have been separated from their component grains. They are seldom quite round. Most of them exhibit one or two flat surfaces where other of the constituents of the compound grains have been attached and are in consequence muller shaped, cap shaped or shortly conical curved on one side and irregular on the other, some are even polygonal. The majority possess a distinct rounded linear or stellate hilum and delicate concentric striations. The largest measure 25 to 35 µm in length the smallest 3 to 15 µm many range from 15µm to 25 µm.

	The granules of Tapioca soften when soaked in water for a few h and preserve their original shape and exhibit a distinct hilum. In many the hilum is stellately fissured, in others the central part of the grain is a translucent mass but the outline is still recognizable, whilst finally many have swollen into a shapeless unrecognizable mass. These are the various stages of gelatinization of the starch by heat in the presence of moisture. The photomicrographs of Cassava starch and tapioca starch are given below  **Arrowroot starch** Arrow root starch is obtained from the roots of *Maranta arundinacea* and other species of *Maranta*. The different varieties are distinguished by their geographical origin. The starch grains are simple and rather large. They are irregular in shape, being rounded, ovoid, pear shaped or sometime almost triangular, the smallest ones are nearly spherical. The largest bear several fine concentric striations and a conspicuous rounded linear or stellate eccentric hilum. The grains average about 30 - 40 µm or even 75 µm as for instance in Bermuda arrowroot the smallest grains vary from 7 – 15 µm The photomicrograph is given below. 
Reference	IS:4706 (Part I) 1978 Methods of test for edible starches and starch products FAO Manuals of Food Quality Control, 14/8, pages 204 – 215
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Moisture in Bakery Products		
Method No.	FSSAI 03.024:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to all varieties of biscuits (sweet, semisweet, crackers, cookies and rusk), cream/filled biscuits and all types of bread.		
Caution	Hot air oven: Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature Exercise extreme caution when opening and closing desiccators		
Principle	The sample is ground. A test portion is dried at 130 ±3 °C to a constant mass. The loss in mass is expressed as a percentage.		
Apparatus/ Instruments	a. Analytical balance, capable of weighing to an accuracy of 0.001 g. b. Grinding mill/Waring blender c. Moisture dishes – made of Aluminium or stainless steel approx 7.5 mm wide and 2.5 mm deep with tight fitting lids d. Force air convection oven –thermostatically controlled to maintain temperature between 105 ± 2 °C. e. Desiccators containing desiccant (Silica gel/P_2O_5, $CaCl_2$).		
Sample preparation	*Powdered or granular substances*: Mix the contents of a whole pack and, if necessary. further grind in a clean and dry mortar to convert it into homogenous powder. Store the around sample in a clean and dry air-tight glass container. *Low moisture crisp products*: For biscuits, cookies and rusks, etc., break the contents of the whole pack into small pieces and subsequently grind the pieces either in an electrically driven, clean dry blender or in a clean and dry mortar to a near homogenous powder. Store the powdered material in a dry air-tight glass container**.** **Note:** Remove the coating/filling if any (cream, caramel, chocolate, marshmallow, jam, jelly, or any other filling between the biscuit) by gentle scraping before powdering the sample. *Semi-moist products*" such as, cakes, bread, buns, etc.: Cut the contents of pack into small pieces with the help of clean dry		

	scissors or a sharp-edged knife and further grind in an electrically driven dry blender taking care that the sample temperature does not rise above45°C in the entire operation. In the case of packs above 400 g, such as bread loaves, slice the uniformly into thin slices 'with the help of a sharp-edged knife and take two slices from the centre and two from each end leaving the outermost end slices and proceed as described above.
Method of analysis	1. Weigh accurately about 5g of the powdered sample in the moisture dish previously dried in an oven and weighed. 2. Place the dish in the oven maintained at 105±2 °C for four h. 3. Cool in the desiccator and weigh. 4. Repeat the process of drying, cooling, and weighing at 30-min intervals until the difference in two consecutive weighs is less than 1 mg. 5. Record the lowest weight.
Calculation	The moisture content, expressed as a percentage by mass of the product, is given by the following equations $$Moisture\ (\%) = \frac{W1-W2}{W1-W} \times 100.$$ Where: W = Mass in g of the empty dish. W1 = Mass in g of the dish with the test portion before drying W2 = Mass in g of the dish with the material after drying
Reference	IS 1011 – 1992 Biscuits – Specification IS 12711:1989 (Reaffirmed Year: 2020) Bakery products – Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Acidity of Extracted Fat in Biscuits, Bread and Bread Type Products		
Method No.	FSSAI 03.025:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to all types of biscuits including, filled and coated, rusk, all types of bread and toasted bread.		
Caution	Petroleum ether is a flammable solvent. Handle with extreme care. Irritating to the eyes and the respiratory tract. Handle only inside a fume hood.		
Principle	Fat is extracted with petroleum ether. The acidity of the extracted fat is titrated with standard potassium hydroxide		
Apparatus/Instrument	a. Soxhlet Apparatus – with a 250 mL flat bottom flask b. Burette (Class A) c. Analytical balance d. Hot air oven		
Chemicals/Reagents	a. Petroleum Ether – Boiling point 40 to 80 °C b. Standard sodium hydroxide solution – 0.05N c. Phenolphthalein Reagent (1.0% in Ethanol (95%) d. 0.0.05 Npotassiumhydroxide solution standardized against potassium hydrogen phthalate. e. Benzene-alcohol-phenolphthalein stock solution		
Preparation of reagents	Benzene-alcohol-phenolphthalein stock solution: To one liter of distilled benzene, add one liter of alcohol or rectified spirit and 0-4 g of phenolphthalein. Mix the contents well,		
Sample preparation	*Powdered or granular substances*: Mix the contents of a whole pack and, if necessary. further grind in a clean and dry mortar to convert it into homogenous powder. Store the around sample in a clean and dry air-tight glass container. *Low moisture crisp products*: For biscuits, cookies and rusks, etc., break the contents of the whole pack into small pieces and subsequently grind the pieces either in an electrically driven, clean dry blender or in a clean and dry mortar to a near homogenous powder. Store the powdered material in a dry air-tight glass container**.** **Note:** Remove the coating/filling if any (cream, caramel, chocolate, marshmallow, jam, jelly, or any other filling between the biscuit) by gentle scraping before powdering the sample. *Semi-moist products*" such as, cakes, bread, buns, etc.: Cut the		

	contents of pack into small pieces with the help of clean dry scissors or a sharp-edged knife and further grind in an electrically driven dry blender taking care that the sample temperature does not rise above 45 °C in the entire operation. In the case of packs above 400 g, such as bread loaves, slice them uniformly into thin slices 'with the help of a sharp-edged knife and take two slices from the centre and two from each end leaving the outermost end slices and proceed as described above.
Method of analysis	1. Weigh accurately approx.20-25g of biscuit/bread powder containing more than 3.0 g of fat and transfer it to the thimble and plug it from the top with extracted cotton and filter paper. **Note**: In case of filled and coated biscuits, the mass of the biscuits includes the filled and coated material also. 2. Dry the thimble with the contents for 15 to 30 min at 100 °C in an oven. Extract the fat with petroleum ether in the Soxhlet apparatus for 8 h 3. Evaporate off the solvent in the flask on a water-bath. 4. Remove the traces of the residual solvent by keeping the flask in the hot air oven for about 30 mins. 5. Cool the flask and add 50 mL of benzene-alcohol mixture. 6. If the test specimen does not dissolve in the cold, connect the flask with a suitable condenser and warm slowly with frequent shaking, until the fat dissolves. 7. Titrate the contents to a distinct pink colour with the standardized potassium hydroxide solution taken in a 10-mLmicro burette. 8. If the contents of flask become cloudy, during titration, add another 50 m1 of the reagent (Phenolphthalein)and continue titration. 9. Make a blank titration of the 50 mL reagent. 10. Subtract the blank titre from the titre of the fat,
Calculation and expression units	Acidity of extracted fat (as oleic acid)% by mass $= \frac{1.41 \times \mathrm{V}}{W1 - W}$ Where: V = volume of 0·05 N potassium hydroxide solution used in titration after subtracting the blank; W1 = mass, in g, of Soxhlet flask containing fat;

	W= mass, in g, of empty Soxhlet flask.
Reference	IS 1011 – 1992 Biscuits – Specification IS 12711:1989 (Reaffirmed Year: 2020) Bakery products – Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Alcoholic acidity (in 90% alcohol) of Bread.		
Method No.	FSSAI 03.026:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to the determination of alcoholic acidity in all types of bread and cornflakes.		
Caution	Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care		
Principle	Alcoholic acidity is defined as mg of NaOH required for 100 g of the sample to have the same alcohol soluble acids. The alcoholic extract of the sample is titrated with standard sodium hydroxide using phenolphthalein as indicator		
Apparatus/Instrument	a. Analytical balance (Accuracy 0.001g) b. Hot air-drying oven c. Burette (Class A)		
Chemicals/Reagents	a. Neutral Ethyl alcohol 90 percent (v/v). b. Standard Sodium hydroxide solution Approximately 0·05N c. Phenolphthalein		
Preparation of reagents	Phenolphthalein-Indicator Solution: 60 mg of phenolphthalein dissolved in 100 mL rectified spirit----		
Sample preparation	a. For semi-moist products" such as, cakes, bread, buns, etc., cut the contents of pack into small pieces with the help of clean dry scissors or a sharp-edged knife and further grind in an electrically driven dry blender taking care that the sample temperature does not rise above 45 °C in the entire operation. b. In the case of packs above 400 g, such as bread loaves, slice them uniformly into thin slices 'with the help of a sharp-edged knife and take two slices from the centre and two from each end leaving the outermost end slices and proceed as described above. c. Cornflakes: Grind in a pestle and mortar about 50 g of the material so that at least 90 % passes through 425 µm IS Sieve. Transfer this prepared sample to a well-stoppered glass bottle for subsequent use d. Dry the sample in a convection oven to remove moisture		
Preparation of Test Samples & Procedure	1. Weigh about 5.0 g of dried (moisture free basis) sample into a stoppered conical flask and add 50 mL of 90% neutral alcohol, previously neutralized against phenolphthalein.		

	2. Stopper, shake and allow to stand for 24 h, with occasional shaking. 3. Filter the alcoholic extract, through a dry filter paper. 4. Titrate the combined alcoholic extract against 0.05 N standard sodium hydroxide solution using Phenolphthalein as an indicator.
Calculation and units of expression	$\text{mL of 1N NaOH required for neutralization of 100 g of sample} = \frac{\text{Titer value} \times \text{Normality of NaOH} \times 100}{\text{Mass of the sample taken}}$
Reference	IS 12711:1989 (Reaffirmed Year: 2020) Bakery products – Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Non–Fat Milk Solids in Milk Bread		
Method No.	FSSAI 03.027:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for estimating non-fat milk solids in Milk Bread		
Caution	Hot air oven: Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature		
Principle	The method is a colorimetric method based on estimating the orotic acid (2, 6·dihydroxypyrimidine-4-carboxylic acid) content. The mean orotic acid content of non-fat milk solids is 62·5 mg/100 g(range 48-0-74-5 mg/100 g)		
Apparatus/Instruments	a. Hot Air oven b. Homogenizer c. Pipettes (Class A): 5, 10 and 25 mL d. Glass stoppered test tubes e. Volumetric flasks (Class A): 10, 50, 100, 500 mL capacity f. Water bath g. Colorimeter/Spectrophotometer		
Chemicals/Reagents	a. Zinc sulphate23 % (m/v) solution b. Potassium hexacyanoferrate 15.0 % (m/v) solution c. *p*- Dimethyl amino benzaldehyde in propanol 3 %(w/v) d. Orotic acid e. Saturated bromine water f. Ascorbic acid solution 10 % g. n- Butyl acetate h. Anhydrous Sodium sulphate i. Whatman filter paper No 541		
Preparation of reagents	a. *Zinc sulphate solution 23 % (w/v):*Dissolve 23 g of Zinc sulphate in 100 mLdistilled water b. *Potassium hexacyanoferrate 15.0 % (m/v*: Dissolve 15.0 g in 100 mL distilled water c. Standard orotic acid – Dissolve 50 mg orotic acid in a mixture of 1 mL of 0.88 ammonia and 10 mL water. Dilute to 500 mL with water. Take 10 mL aliquot and dilute to100 mL with water. Further dilute 2.5, 5, 10, and 15 mL of this solution to 50 mL to produce solutions containing 2.5, 5, 10,15 µg of orotic acid per 5 mL.		
Sample preparation	Use dried bread powder after determining the moisture content		

Preparation of Test Samples& Procedure	1. Transfer 5 g of dried sample obtained after determination of moisture, to the homogenizer, add 100 mL water and mix at the maximum speed for one minute. 2. Filter the supernatant liquid through a 15 cm Whatman filter paper No 541 or equivalent, rejecting the first 10 mL. Only 5 mL is required for the determination. 3. Into a series of glass stoppered tubes, pipette a) 5 mL of test solution (containing 2 - 15 µg orotic acid), b) 5 mL of each of the standard orotic acid solutions and c) 5 mL of water to act as a blank. 4. Add 1.5 mL of saturated bromine water to each tube and allow the mixture to stand at room temperature for not more than 5 minutes. 5. As the addition of bromine water is made to the series of tubes, the times will vary slightly between each, the time of reaction is not critical provided it is between 1 and 5 mins. 6. Add 2 mL of 10 % Ascorbic acid solution to each tube and place the tubes in a water bath at 40 °C for 5 minutes. 7. Cool to room temperature, add to each tube 4 mL n-butyl acetate and shake vigorously for 15 seconds. 8. Transfer the upper separated layers to dry test tubes containing 1 g anhydrous Sodium sulphate. Mix gently. Add another gram of anhydrous Sodium sulphate. Mix gently and allow to separate. 9. Transfer the clear butyl acetate layer to 1 cm cell and measure the optical density/absorbance at 461 - 462 nm against the blank.
Calculation	Draw a calibration graph of the standard orotic acid solution versus the absorbance. Carry out a regression analysis and obtain the equation y=mx+c Determine the orotic acid content in 5 mL of sample extract by interpolation of the absorbanceof the sample. Convert assuming that skim milk powder contains 62.5mg orotic acid per 100 g.
Reference	IS 12711:1989 (Reaffirmed Year: 2020) Bakery products – Methods of Analysis Pearson Composition and Analysis of Foods 9th edn, page 316
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Ash excluding Sodium Chloride in Cornflakes and Custard Powder.		
Method No.	FSSAI 03.028:2022	**Revision No. & Date**	0.0
Scope	This method is applicable for the determination of total ash excluding sodium chloride in cornflakes, custard powder edible starches and starch products (e.g. Tapioca Sago and Palm sago starch)		
Caution	Muffle furnace: When using the Muffle furnace, it is essential to wear the protective gloves provided, as well as lab coat (with sleeves rolled down) and safety glasses. Use tongs provided for loading/unloading the furnace. Practice using the tongs before attempting to pick up a precious or extremely hot sample. Nitric acid is highly corrosive and can cause irritation to the eyes, skin, and mucous membrane. Always add acid to water to prevent splattering from overheating and boiling. Clean-up spills promptly with appropriate materials. Handle only inside a fume hood		
Principle	The sample is ashed in a muffle furnace and total ash determined. The sodium chloride content is determined by precipitation with excess silver nitrate. The excess silver nitrate is determined by back titration with ammonium thiocyanate.		
Apparatus/Instruments	a. Burette (Class A) b. Hot air oven c. Muffle furnace d. Analytical balance (Accuracy 0.001g)		
Chemicals/Reagents	a. Silver nitrate b. Ammonium thiocyanate c. Concentrated Nitric acid d. Ferric alum e. Whatman No 1 Filter paper		
Preparation of reagents	a. Standard Silver nitrate – 0.1 N b. Ammonium thiocyanate solution - standardized against 0.1 N Silver nitrate solution c. Dilute Nitric acid – 1 + 9: To 900 mL of water add 100 mL of concentrated Nitric acid d. Concentrated Nitric acid – 4+ 1 To 100 mL water add 400 mL concentrated Nitric acid e. Ferric alum indicator solution – Saturated solution of ferric alum in water		

Sample preparation	Cornflakes: Grind about 50 g in a pestle and mortar so that at least 90 % passes through 425-µm IS Sieve. Transfer this prepared sample to a well-stoppered glass bottle for subsequent use
Method of analysis	1. Ignite the dried material in a dish over a Bunsen burner for about one h. Complete the ignition by keeping in a muffle furnace at 600 ± 20 °C until "grey ash results. 2. Cool in a desiccator and weigh. 3. Heat and dish again in the muffle furnace at 600 ± 20 °C for 30 min and cool in the desiccator. 4. Repeat this process of heating, cooling and weighing until the difference between two successive weighing is less than one milligram. Note the lowest mass. 5. Dissolve the ash in 25 mL of the dilute nitric acid solution (1:9). Filter through "Whatman filter paper No.1 or its equivalent, collecting" the filtrate in a 100 mL volumetric flask, and wash the contents thoroughly with hot water. 6. Make the volume to 100 mL 7. To a 25-mL aliquot of the filtrate, add excess of the standard silver nitrate solution (20 mL). stirring well to flocculate the precipitate of silver chloride. 8. Filter and wash the precipitate thoroughly with water. 9. To the combined filtrate, add 5 mL of each of the ferric alum indicator 10. solution and concentrated nitric acid solution (4: 1). 11. Titrate the, excess of silver nitrate with the standard ammonium thiocyanate solution to astable light brown color end point persists
Calculation	$\text{Total ash on dry wt basis} = \frac{(W2 - W)}{(W1 - W)} \times 100$ Where: W = Mass in g of empty dish. W1 = Mass in g of dish with dried sample W2 = Mass in g of dish with the ash $\text{NaCl on dry mass basis(percent by mass)} = \frac{(V1N1 - V2N2 \times 5.85}{(W1 - W)} \times \frac{V3}{V4}$ Where: V1 = vol of standard silver nitrate added initially N1 = Normality of silver nitrate solution V2 = vol of standard ammonium thiocyanate used for titrating excess silver nitrate

	N2 = Normality of standard ammonium thiocyanate solution W1 = mass in g of dish with sample W = mass in g of empty dish V3 = volume in mL to which the filtrate was made up V4 = Volume in mL of the aliquot taken for titration. Total ash excluding sodium chloride = Total ash on dry wt. basis – Sodium chloride on dry mass basis
Reference	IS: 1158, 1973 (Reaffirmed Year: 2010) Specification for cornflakes IS: 4706 (Part II) – 1978 (Reaffirmed 2005) Indian Standard methods of test for edible starches and starch products Part ii Chemical methods
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Solubility in Malted Milk Foods		
Method No.	FSSAI 03.029:2022	**Revision No. & Date**	0.0
Scope	The method for solubility is extendable to malted milk food, infant food powders.		
Caution	Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature.		
Principle	The sample is shaken with water and the total solids of the suspension determined before and after centrifuging. The amount of powder remaining in suspension after centrifuging expressed as a percentage of the total amount in suspension is taken as the measure of solubility.		
Apparatus/Instruments	a. 50 mL centrifuge tubes b. Spoon-shaped spatula c. Hot air oven d. Analytical balance (Sensitivity 0.001 g)		
Chemicals/Reagents	Distilled water		
Sample preparation	a. Weigh accurately 4 g of the material into a 50 mL boiling tube, and add 32 mL of water warmed to 50±1 °C. Shake the tube for 10 seconds. Place the tube in a water bath maintained at 50±1 °C for 5 minutes and shake the tube for one minute. b. Fill the reconstituted milk into a 25 mL centrifuge tube and centrifuge for 10 minutes at 770× g. c. Cool in a refrigerator or in ice until the fat solidifies (taking care that milk does not freeze). d. Remove the fat layer with spoon shaped spatula. e. Bring the milk to room temperature (27±l °C). f. Break up the deposit with a glass rod. Cork the tube and shake vigorously until the liquid is homogenous		
Method of analysis	**Determination of Total Solids** 1. Pipette 2 mL of homogenous liquid in a previously dried and weighed aluminum dish provided with a tight-fitting lid and weigh (No. 1). 2. Centrifuge the tube for 10 min at 770 ×g for 10 min. 3. Without disturbing the sediment, pipette 2 mL of the		

	supernatant into a second dish (No. 2) and weigh. 4. Remove the lids of both the dishes (No. 1 and 2) and place on a water bath till the sample is dry. 5. Keep the dishes in air oven at 98±2 °C for 90 min, 6. Cool in a desiccator and weigh. 7. Repeat heating and weighing till constant weight is obtained (within 2 mg).
Calculationand expression of units	Solubility (%) $= \frac{W4 \times W1}{W3 \times W2}$ Where: W1 = Mass of liquid taken in dish No. 1 before centrifuging W2 = Mass of liquid taken in dish No. 2 after centrifuging W3 = Mass of total solids remaining after evaporation of dish No. 1 W4 = Mass of total solids remaining after evaporation of dish No. 2
Reference	FAO Manuals of Food Quality Control 14/8 page 31 British standard 1743: Part 2: 1980
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Cocoa Powder in Malted Milk Foods		
Method No.	FSSAI 03.030:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for malted milk foods		
Caution	*Chloroform*: Handle with care. Chloroform is highly toxic and is a probable human carcinogen. Avoid contact with eye and skin. Exposure to chloroform over a long period of time may damage liver and kidneys. Large amounts of chloroform can cause sores when chloroform touches the skin. Handle only inside a fume hood. *Concentrated Sulphuric acid*: Concentrated sulphuric acid is corrosive and can cause severe burns. Handle with care. Always add concentrated acid to water and not water to acid.		
Principle	The method is based on the extracted alkaloids of cocoa. The alkaloid is extracted with aqueous ethanol (80%) and magnesium oxide. The extract is clarified with lead acetate and concentrated and extracted into chloroform. The alkaloid content is estimated by estimating the nitrogen content by Kjeldahl method and using a conversion factor.		
Apparatus	a. Air condenser b. Buchner Funnel c. Separatory Funnel d. Distillation Assembly – identical with Nitrogen estimation – The assembly consists of a round bottom flask of 1000 mL capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube. The other end of the connecting bulb tube is connected to the condenser which is attached by means of a rubber tube to a dip tube which dips into a known quantity of standard sulphuric acid contained in a 250 mL flask. e. Volumetric flask f. Kjeldahl flask g. Water bath		
Materials and Reagents	a. Concentrated Sulphuric acid - Approx 98 % b. Dilute alcohol – 80 % (v/v) c. Potassium Ferrocyanide -. d. Sodium Hydroxide solution – 50% e. Standard Sulphuric acid solution – 0.1 N f. Standard Sodium hydroxide solution – 0.1 N g. Methyl red indicator h. Zinc Acetate solution		

	i. Magnesium oxide j. Standard Hydrochloric acid – 10 % k. Sucrose anhydrous, pure l. Selenium
Preparation of reagent	a. Potassium Ferrocyanide -Dissolve 10.6 g of crystallized Potassium Ferrocyanide in water and make up to 100 mL. b. Methyl red indicator – Dissolve 1 g Methyl red in 200 mL of 95 % alcohol c. Zinc Acetate solution – Dissolve 21.9 g of crystallized Zinc acetate and 3 mL glacial acetic acid in water and make upto 100 mL
Method of analysis	1. Grind 20 g of the material to a smooth paste with a little alcohol and transfer to a 200 mL flask with more of the same alcohol to make about 100 mL. 2. Add 1 g of freshly ignited Magnesium oxide and digest on a boiling water bath for 1½ h using an air condenser and shaking occasionally. 3. Filter while hot through a Buchner funnel, return the residue to the flask and digest again for 30 minutes with 50 mL of alcohol. 4. Filter and repeat the digestion once more. 5. Evaporate the combined filtrate on a steam bath adding hot water from time to time to replace the alcohol lost. When all the alcohol is lost finally concentrate to about 100 mL. 6. Add 2- 3 mL of concentrated HCl and transfer the liquid to a 200 mL volumetric flask. 7. Cool, add 5 mL of Zinc acetate, mix and add 5 mL of potassium ferrocyanide solution. 8. Make up to mark and mix thoroughly. Allow the flask to stand for few minutes and filter through a dry filter paper. 9. Evaporate the whole of the filtrate to about 10 mL, transfer to a separatory funnel, and extract with five successive 30 mL portions of chloroform, with vigorous and thorough shaking. 10. Wash the combined extracts with 3-5 mL water. Repeat the process of extraction with five more successive portions of chloroform, wash the second chloroform extract with the same wash water used before, combine all the extracts and distill the chloroform. 11. Dissolve the residue in a little hot water, transfer to a Kjeldahl flask, and add 0.2 g sucrose and 10 mL of concentrated sulphuric acid. Heat over a small flame until frothing ceases, add 0.2 g selenium and digest until colourless. 12. Cool the contents of the flask. Transfer quantitatively to the round bottom flask with water, the total quantity of water used to be 200 mL.

	13. Add with shaking a few pieces of pumice stone to prevent bumping. Add about 50 mL of Sodium hydroxide (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. 14. Assemble the apparatus taking care that the tip of the condenser extends below the surface of standard sulphuric acid contained in the flask. 15. Mix the contents of the flask by shaking and distill until all the ammonia has passed over into standard sulphuric acid. 16. Reduce the burner flame. 17. Detach the flask from the condenser and shut off the burner. 18. Rinse the condenser thoroughly with water into the flask. 19. Wash the tip carefully so that all traces of condensate are transferred to the flask. 20. When all the washings have drained into the flask, add 2- 3 drops of methyl red indicator and titrate with standard sodium hydroxide solution. Carry out a blank determination using all reagents in the same quantities but without the sample under test.
Calculation	First calculate alkaloid by multiplying nitrogen content by factor 3.26. Cocoa powder in the material is then calculated on the assumption that the average value of total alkaloids in cocoa powder is 3.2 % using following formula $$\text{Cocoa powder \% by mass} = \frac{228.2\ (\text{B} - \text{A})\ \text{N}}{W}$$ Where: B = Volume in mL of standard Sodium hydroxide used to neutralize the acid in the blank determination A = Volume in mL of standard Sodium hydroxide used to neutralize excess of acid in the test with material. N = Normality of standard Sod hydroxide solution W = mass in g of the material taken for the test
Reference	Moir, D. D., and Hinks, E. (1935) The determination of total alkaloid in cocoa and of cocoa- matter in flour confectionery. Analyst, 712, 439-447
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Synthetic Colour in Biscuits, Cakes and Other Bakeryware.		
Method No.	FSSAI 03.031:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to the estimation of synthetic colors in all bakery items		
Caution	*Hydrochloric acid*: Handle with extreme care. Concentrated HCl is corrosive. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood.		
Principle	The acid dyes in food are dissolved in ammoniacal alcohol followed by acidification and adsorption of the dyes from the solution on pure wool. The adsorbed dye is stripped and subjected to paper chromatography.		
Apparatus/Instruments	a. Glass pestle and mortar b. Beakers 100- and 250-mL capacity c. Chromatographic Chamber 30 cm x 20 cm 0 10 cm d. Test tubes e. Spectrophotometer f. Water bath g. Porcelain dish h. Chromatographic paper Whatman No 1 or equivalent		
Chemicals/reagents	a. Concentrated hydrochloric acid b. Ammonia c. 100% pure knitting wool		
Preparation of reagents	a. 0.1 N Hydrochloric acid: 8.5 mL of concentrated HCl diluted to 1 L with water b. 100 % pure wool (white knitting)–Boil in 1 % sodium hydroxide solution and then in water to remove alkali. Wash repeatedly with distilled water and dry. c. 2 % ammonia in 70 % alcohol		
Sample preparation	Grind the sample to a fine powder		
Method of analysis	**I. Extraction of dye** 1. Grind 10 g of sample thoroughly with 50 mL of 2 % ammonia in 70% alcohol, and allow it to stand for an hour and centrifuge.		

	2. Pour the separated liquid into an evaporating dish and evaporate on water bath. Take up the residue in 30 mL dilute acetic acid. 3. Add a 20 cm strip of pure white wool to the solution and boil. When the wool takes up the colour fairly completely, take out and wash with water. 4. Transfer the washed wool to a small beaker and boil gently with dilute ammonia (1+4). If the colour is stripped, the presence of an acid dye is indicated. 5. Remove the wool. Make the liquid slightly acidic and add a fresh piece of wool and boil until the colour is removed. Extract the dye from the wool again with a small volume of dilute ammonia. This double stripping technique usually gives a pure colour. 1. Natural colour may also dye the wool during the first treatment but the colour is not removed by ammonia. 2. Transfer the solution to a volumetric flask and make the volume to 50 mL with water. Note: - Basic dyes can be separated by making the food alkaline with ammonia, boiling with wool and then stripping with dilute acetic acid. All the present permitted water soluble colours are acidic and the presence of a basic dye would indicate presence of non-permitted dye. Note: The method given is sensitive only beyond 20-25 ppm.
	II. Separation of Colours by Paper Chromatography 1. Take a Whatman No 1or equivalent filter paper sheet (15 cm x 30 cm) and draw a line parallel to the bottom edge of the sheet about 2 cm away 2. Spot 0.5 mL of extracted dye with the help of a graduated pipette and apply it on the filter paper in the form of a band on the line. 3. Prepare 0.1% solutions of permitted dyes as reference standards and apply spots of all these dyes on the line ~1.5 cm between two spots. *Note*: The R_f values vary slightly owing to variation of temperature, solvent purity and solvent saturation of the chromatographic chamber. It is thus essential that known dyes are applied with the sample as control 4. Allow the spots to dry and subsequently suspend the paper sheet in the chromatographic chamber such that the lower edge

	of the sheet remains dipped in the solvent placed in the chamber. 5. The following solvent systems may be used for separation of dyes. Solvent 5 has been found to give good resolution. I. 1% ammonia = 1 mL ammonia (sp gr 0. 88) + 99 mL water II. 2.5% Sodium chloride III. 2% Sodium Chloride in 50% alcohol IV. Isobutanol: Ethanol: Water (1: 2: 1 (v/v)) V. n-Butanol: Water: Acetic acid (20: 12: 5) VI. Isobutanol: Ethanol: Acetic acid (3: 12: 5) 6. Close the chromatographic chamber tightly and let the solvent rise. 7. When the solvent front has moved about 20 cm away from the base line, remove the filter paper sheet and allow it to air dry. 8. Mark coloured bands and carefully cut the coloured strips from the paper. Cut the coloured strips into small pieces and transfer to a test tube and add about 1 mL 0.1 N HCl. 9. Allow the colour to be extracted 10. Decant the coloured extract into a volumetric flask. 11. Repeat the process of extraction and decanting till all the colour is extracted from the paper. Make up the volume. 12. Determine absorbance maxima and read at the absorbance maximum against a blank prepared by cutting an equivalent strip plain portion of chromatogram and extracting it with 0.1 N HCl. 13. From the absorbance values compute the concentration of the dye by reference to the plot of concentration versus optical density. **III. Calibration curve** 1. Prepare 0.1% solution of the dye in 0.1 N HCl. Take 0.25, 0.50, 0.75, 1.0, 1.25- and 1.5-mL aliquot of this and dilute to 100 mL with 0.1 N HCl. 2. Read the absorbance at respective absorbance maxima. 3. Plot absorbance values against concentration of the dye. From the regression line calculate the amount of dye

The Rf values and absorbance maxima of the permitted water-soluble dyes are given below which may be used as a guide in characterization of the dye and in determining their concentration. Chromatographic Rf values are known to vary because of variation in temperature, solvent purity and solvent -saturation of the chromatography chamber, It is, therefore, essential that known dyes should be applied along with the sample for comparison of Rf values under actual conditions used in the test

Solvent System	*Rf Values*						*Absorba* *Maximum*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Ponceau 4 R	0·95	0·36	0·42	0·29	0·33	0 29	505
Carmoisine	0·61	0·04	0·56	0·51	0·56	0·28	515
Amaranth	0·77	0·06	0·20	0·24	0·19	0·20	520
Erythrosine	0·23	0·00	0·70	1·00	1·00	0·38	525
Fast red E	0·45	0·00	0·60	0·45	0·54	0·60	505
Sunset yellow FCF	0·78	0·26	0·65	0·49	0·45	0·56	480
Tartrazine	1·00	0·26	0·30	0·26	0·18	0·22	430
Indigo carmine	—	0·07	0·30	0·28	0·21	0·27	615
Brilliant blue FCF	—	—	—	—	—	—	630
Green S	1·00	0·88	1·00	0·73	0·57	0·50	635
Green FCF	—	—	—	—	—	—	624

The solvent systems are 2) 1% ammonia = 1 mL ammonia (sp gr 0. 88) + 99 mL water3) 2.5% Sodium chloride, 4) 2 % Sodium Chloride in 50% alcohol, 5) Isobutanol: Etahnol : Water (1: 2: 1 (v/v)), 6) n-Butanol : Water : Acetic acid (20 : 12: 5) and 7) Isobutanol :Ethanol : Acetic acid (3 : 12 : 5)

Calculation and units of expression	From the absorbance values of the sample compute the concentration of the dye by referring to the regression line (y=mx+c plot) of concentration versus absorbance
Reference	IS 12711: 1989 Bakery Products – Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Residual Hexane in Solvent Extracted Oilseed Flours		
Method No.	FSSAI 03.032:2022	**Revision No. & Date**	0.0
Scope	This is a method for the determination of total amount of volatile hydrocarbons, referred to as Hexane remaining in oilseed residues after extraction with hydrocarbon-based solvents. The method is applicable to all oilseed and legume flours, textured soybean products		
Caution	Avoid use of plastic containers.		
Principle	Desorption of Hexane by heating at 110 °C with water in a closed vessel, and determination of the hexane in the headspace by gas chromatography using capillary or packed columns and expressing the results as Hexane.		
Apparatus/Instruments	a. Gas Chromatograph equipped with flame ionization detector, recorder and integratorwith Glass capillary column approx 30 m long and 0.3 mm in diameter coated with methyl polysiloxane film of 0.2 µm thickness or a packed column of at least 1.7 m length with 2-4 mm internal diameter packed with acid washed diatomaceous earth of particle size of 150 – 180 µm and coated with methyl polysiloxane. If a capillary column is used the apparatus shall have a 1/100 input divider. b. Electric oven: capable of being maintained at 110 °C c. Gas Syringe: graduated capacity 1 mL, preferably with a valve d. Penicillin type flasks of capacity 50- 60 mL all with the same volume to within 2 %. e. Septa, inert to solvents, of approximately 3 mm thickness capable of producing a hermetic seal after crimping. f. Metallic foil caps of Aluminum g. Crimping pliers h. Liquid syringe 10 µL capacity.		
Reagents Chemicals	(a) Standard: n-Hexane or light petroleum, with a composition similar to that used in the industrial extraction of oilseeds, failing that n – Hexane. (b) Carrier Gas: - Hydrogen or Nitrogen, Helium etc, dry and containing less than 10 mg/kg of Oxygen. (c) Auxiliary gases (1) Hydrogen 99.9 % pure, containing no organic impurities (2) Air containing no organic impurities		
Preparation of reagents	It is essential that the loss of hexane from sample be prevented. The sample shall fill a completely sealed container (preferably a crimped metal box and		

	shall be stored at – 20 °C below in a deep freezer. Plastic containers shall not be used. The determination of residual Hexane shall be carried out as soon as the container has been brought to room temperature and opened.
Method of analysis	Sample Analysis 1. Set the oven temperature of the GC at 40 °C, injector and detector temperature at 120 °C, Carrier gas pressure at 0.3 bar (30 kPa). 2. Weigh to the nearest 0.1 g, 5 g of the laboratory sample into a flask. Add 2.5 mL water; seal the flask with a septum, cover with a foil cap and crimp with the pliers. All these operations should be performed rapidly. 3. Place the flask in the oven maintained at 110 °C for 90 minutes, remove the flask from the oven and let it cool for 2 minutes. Agitate by inverting. It is important to leave the flasks in the oven for the same length of time for each sample. 4. Using the gas syringe previously heated to 50 – 60 °C take exactly 0.5 mL of gaseous phase and inject quickly into the GC. 5. Carry out three determinations for each sample. **Construction of calibration curve** 1. Three points with 2.5, 5.0, 10.0 µL of standard solvent are usually sufficient to construct the calibration curve, they correspond to 264, 660, 1320 mg/kg of Hexane if the test portion is 5 g. 2. Prepare a calibration series using flasks of the same capacity as used for the determination. 3. Add to the flasks 6 mL* of water followed immediately by various quantities of n–Hexane measured accurately with the help of the syringe. 4. Seal each flask with the septum, cover with the foil cap and crimp with the plier place the various flasks for the establishment of one calibration graph in the oven for 15 minutes at 110 °C. 5. At the end of this time remove the flasks from the oven and leave to cool for 2 minutes. 6. With the gas syringe heated between 50 – 60 °C take exactly 0.5 mL of take exactly 0,5 mL of the gaseous phase (headspace) and inject quickly into the chromatograph. Carry out two determinations on the sample. * 5 g of hydrated residue per 2.5 mL of water occupies on average a volume of 6 mL.
Calculation and units of expression	1. Construct the calibration graph by plotting the area under the curve of the solvent peak as a function of the mass of the solvent introduced into the flask (1 µL corresponding to 660 µg).

	2. Determine the sum of the peak areas of Hexane and various Hydrocarbons which usually make up the technical solvent. 3. Note: Do not include peaks due to oxidation products if present in significant amounts but report calibration these separately. Read off from the graph the mass $m_1$in microgram of Hexane present in the flask The total residual Hexane in the residue expressed in microgram of hexane per kilogram= $\frac{M1}{M0}$ Where: M_0 = the mass in g of the test portion. M_1 = the mass in microgram of solvent present in the flask. Take as the result the arithmetic mean of three determinations.
Reference	IS 12983: 1990/ISO 8892:1987 (Reaffirmed 1998), Oilseed Residues – Determination of Total Residual Hexane Dupuy H.P. Fore, S.P., and Rayner, E.T. (1975)"Rapid Quantitative Determination of Residual Hexane in Oils by Direct Gas Chromatography," published in the "Journal of the American Oil Chemists' Society," 52, 118-120,
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Oxalic Acid in Solvent Extracted Sesame Flour		
Method No.	FSSAI 03.033:2022	**Revision No. & Date**	0.0
Scope	This method shall apply only to edible flour obtained from white sesame seeds.		
Caution	Hydrochloric acid: Handle with extreme care. Concentrated HCl is corrosive. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood. Ammonium hydroxide: Handle with extreme care. Avoid contact with eyes and skin. Eye contact may result in eye burns and temporary loss of sight. If inhaled, mild exposure can cause nose irritation. Handle only inside a fume hood. Concentrated sulphuric acid is corrosive and can cause severe burns. Handle with care. Always add concentrated acid to water and not water to acid.		
Principle	Proteins are precipitated with phosphor-tungstic acid. The oxalic acid in the sample is precipitated as calcium oxalate. Oxalate is precipitated as calcium oxalate from a buffered (pH 4.0-4.5) solution. The precipitate is separated by centrifugation. The oxalic acid is determined by titrating the oxalate in the precipitate with potassium permanganate.		
Apparatus	a. Waring Blender b. Burette (Class A) c. Volumetric flask (Class A): 500 mL		
Chemicals	a. Dilute Hydrochloric Acid (1+ 1) b. Ammonium hydroxide solution – sp gr 0.880 c. Concentrated sulphuric acid d. Potassium permanganate solution – 0.02 standardized with oxalic acid e. Capryl alcohol f. Calcium chloride		
Preparation of reagents	a. Phosphoric tungstate reagent – Dissolve 24 g Sodium Tungstate in water. To this add 40 mL of syrupy phosphoric acid (sp gr 1.75) and dilute the solution to one L b. Calcium chloride buffer solution – Dissolve 25 g of anhydrous Calcium chloride in 500 mL of 50 % glacial acetic acid and add this solution to a solution of 530 g of Sodium acetate in water, diluted to 500 mL c. Dilute Hydrochloric acid (1+ 1): Dilute concentrated HCl 1:1 with distilled water d. Sulphuric acid -10% solution: Dilute 20 mL of concentrated Sulphuric		

	acid with 180 mL of distilled water. e. Wash solution – A 5% solution of acetic acid kept over calcium oxalate at room temperature. Shake the solution periodically and filter before use.
Method of analysis	1. Homogenize about 6 g of the sample with about 100 mL water in the blender 2. Transfer the mixture to a 600 mL beaker with the minimum number of washings. 3. Add 2 volumes of dil HCl to each 10 volumes of liquid (to give an approx normal concentration) 4. Add one or two drops of capryl alcohol and boil for 15 minutes. 5. Allow to cool, transfer to a 500 mL volumetric flask, dilute to mark and after an occasional shaking set it aside overnight. 6. Mix and filter through a dry filter paper. 7. Transfer by means of a pipette 25 mL of filtrate into a tube fitted with a stopper. 8. Add 5 mL of phosphoric tungstate reagent, mix by inverting once or twice and set the mixture aside for 5 h. 9. Centrifuge for 10 min at 15,000 × g (3000 rpm with 150 cm radius) 10. Transfer exactly 20 mL of clear solution to a 50 mL centrifuge tube and add ammonium hydroxide drop wise from a burette until the solution is alkaline as indicated by formation of a slight precipitate of phospho-tungstate. 11. Add 5 mL of Calcium chloride reagent, stir with a fine glass rod and leave the tube overnight in a refrigerator at 5 – 7 °C. 12. Centrifuge for 10 minutes, carefully remove the washings 13. Dissolve the precipitate in 5 mL of 10 % sulphuric acid, place the tube in a water bath at 100 °C for 2 minutes and titrate the oxalic acid with standardized 0.02 N Potassium permanganate.
Calculation	1 mL of 0.02 N Potassium permanganate = 0.00090g oxalic acid
Reference	IS specification No IS 6108 - 1971 Specification for Edible Sesame Flour (solvent extracted) Franco and Krinitz, (1973) Determination of Oxalic Acid in Foods. J. AOAC, 56,164-166
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Free Gossypol in Cotton Seed Flour		
Method No.	FSSAI 03.034:2022	**Revision No. & Date**	
Scope	The term free gossypol defines gossypol and gossypol derivatives in cottonseed products which are soluble in aqueous acetone under the conditions of the method. The method is applicable to full-fat cottonseed, cottonseed meals, or expanded collets and solvent-extracted cottonseed meal that contain Free gossypol (FG) within the ranges of 0.02–0.25% and 0.9–1.8%.		
Principle	This method for estimating free gossypol (FG) consists of adding water and acetone separately to a fixed sample weight, mixing, filtering, diluting with 65% acetone, and reading absorbance on a spectrophotometer		
Apparatus/Instruments	a. Mechanical shaker to hold 250 mL Erlenmeyer flasks and provide vigorous shaking b. UV-Vis Spectrophotometer or colorimeter equipped with a filter having maximum transmittance between 440- 460 nm c. Grinding mill- with 1 mm screen d. Glass beads about 6 mm diameter e. Erlenmeyer flasks 250 mL, f. Whatman No 2 or equivalent g. Volumetric flasks 25, 200 ,250 mL, Class A h. Water bath for operation at 100 °C equipped with clamps for supporting 25 mL volumetric flasks		
Reagents/Chemicals	a. Acetone b. Isopropyl alcohol (2-propanol c. Aniline – distilled over a small amount of Zinc dust. Redistill when the reagent blank exceeds 0.022 absorbance (95 % transmittance) d. Thiourea e. Concentrated Hydrochloric acid f. Gossypol - Primary standard or gossypol acetic acid (89.61 % gossypol by wt) to be used for calibration		
Preparation of reagents	a. Aqueous acetone – Mix 700 mL acetone with 300 mL distilled water. b. Aqueous Isopropyl alcohol (2-propanol) – Mix 800 mL isopropyl alcohol with 200 mL water. c. Thiourea solution – Dissolve 10 g thiourea in water and make up to 100		

	mL d. 1.2 N HCl –Dilute 106 mL concentrated HCl (35-37%) to 1 L with water e. Standard Gossypol solution 1. Accurately weigh 25 mg primary standard gossypol or 27.9 mg gossypol acetic acid and transfer quantitatively to a 250 mL volumetric flask using 100 mL of acetone. 2. Add 1 mL glacial acetic acid, 75 mL water, dilute to volume with acetone and mix well. 3. Pipette 50 mL of this solution into a 200 mL volumetric flask, add 100 mL acetone, 60 mL water and dilute to volume with acetone. Mix well. 4. This standard gossypol solution contains 0.025 mg of gossypol per mL if exactly 25 mg gossypol or 27.9 mg of gossypol acetate were weighed. It is stable for 24 h when protected from light
Method of analysis	1. Grind about 50 g sample in a Wiley grinding mill to pass 1 mm screen. 2. The weight of the sample and the aliquot of the acetone extract to be taken for test shall depend on the gossypol content but sample size should not exceed 2-5 g if the free gossypol is expected to be between 0.2 – 0. 5% and the aliquot of extract to be taken for test should be 10 mL 3. Transfer the accurately weighed sample to a 250 mL Erlenmeyer flask, add a few glass beads and 50 mL aqueous acetone, stopper and shake vigorously on a mechanical shaker for 1 h. Filter through a dry filter paper discarding the first 5 mL and collect filtrate in a small flask. Pipette duplicate aliquots into 25mL volumetric flasks. 4. To one sample solution designated as solution A, add 2 drops of 10 % aqueous thiourea, 1 drop of 1.2 N HCl and dilute to volume with aqueous isopropyl alcohol. 5. To the second sample designated as solution B, add 2 drops of 10 %, aqueous thiourea, 1 drop of 1.2 N HCl and 2 mL of redistilled aniline. A rapid delivery pipette may be used for dispensing aniline. 6. Prepare a reagent blank containing a volume of aqueous acetone solution equal to that of the sample aliquot and add 2 drops of 10% thiourea and 2 mL of aniline (do not add any 1.2 N HCl). 7. Heat the sample aliquot B and the reagent blank in a boiling water bath for 30 minutes. 8. Remove the solutions from the bath, add about 10 mL of aqueous isopropyl alcohol; to effect homogeneous solution and cool to room temperature. Dilute to volume with aqueous isopropyl alcohol 9. Determine the absorbance of sample aliquot A at 440 nm using aqueous isopropyl alcohol to set the instrument at zero absorbance (100%

	transmittance). 10. With the instrument at zero absorbance with aqueous isopropyl alcohol, determine the absorbance of reagent blank. If the reagent blank exceeds 0.022 absorbance units, the analysis must be repeated using freshly distilled aniline. 11. Determine the absorbance of sample aliquot B at 440 nm using the reagent blank to set instrument at 0 absorbance. 12. Calculate the corrected absorbance of the aliquot as mentioned below in calculation column. Corrected absorbance = (absorbance of B – absorbance of A)
Calibration curve	1. Prepare a calibration curve by taking1, 2, 3, 4, 5, 7 , 8, 10 mL aliquot of standard gossypol solution (0.025 mg/mL) into 25 mL volumetric flask. 2. To one set of aliquots designated C add 2 drops of 10 % aqueous thiourea, 1 drop of 1.2 N HCl and dilute to volume with aqueous isopropyl alcohol and determine its absorbance. 3. To the other set of aliquots designated D add 2 drops of aqueous thiourea, 2 drops of 1.2 N HCl and 2 mL of redistilled aniline. 4. Prepare a reagent blank containing 10 mL of aqueous acetone, 2 drops of aqueous thiourea and 2 mL of aniline (do not add HCl). 5. Heat the standards designated as D and the reagent blank in boiling water bath for 30 minutes, cool and dilute to volume with aqueous isopropyl alcohol and determine their absorbance. 6. Determine corrected absorbance = (absorbance of D – absorbance of C) 7. Plot the corrected absorbance for each gossypol standard against mg of gossypol in 25 mL volume to obtain the calibration graph and carry out a regression analysis
Calculation	Corrected absorbance for sample = (absorbance of B – absorbance of A) From the corrected absorbance of the sample, determine the mg of gossypol in the sample aliquot by reference to the regression line y=mx+c generated as described above
Reference	AOCS (1989) Official Method Ba 8 – 78
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Gossypol		
Method No.	FSSAI 03.035:2022	**Revision No. & Date**	0.0
Scope	This method is applicable to full-fat cottonseed, cottonseed meals, or expanded collets and solvent-extracted cottonseed meal that contain both free and bound gossypol		
Principle	Gossypol and gossypol derivatives both free and bound in cottonseed products which are capable of reacting with 3 - amino -1 propanol in dimethylformamide solution to form diaminopropane complex, which then reacts with aniline to form dianilino-gossypol under the conditions of the method.		
Apparatus/Instrument	a. Mechanical shaker to hold 250 mL Erlenmeyer flasks and provide vigorous shaking b. Spectrophotometer equipped with cells of 1 cm light path or colorimeter equipped with a filter having maximum transmittance between 440-460 nm c. Grinding mill- with 1 mm screen d. Glass beads about 6 mm diameter e. Erlenmeyer flasks 250 mL f. Filter paper, medium retention, 11 cm size (Whatman No 2 or eqvt) g. Volumetric flasks 25, 200, 250 mL, Class A h. Water bath set at 100 °C equipped with clamps for supporting 25 mL volumetric flasks i. Pipettes (Class A) 1, 2, 4, 5, 10 mL.		
Chemicals/Reagents	a. Isopropyl alcohol b. n – Hexane (b.p 68-69 ^{0}C), c. Dimethyl formamide, d. 3 – amino 1 propanol (propanolamine), free of colour, e. Glacial acetic acid f. Aniline. The aniline should be redistilled over zinc dust using water cooled condenser		
Preparation of reagents	(1) Isopropyl alcohol- hexane mixture (60 + 40) (2) Complexing reagent prepared by pipetting 2 mL of 3 amino-1 propanol and 10 mL glacial acetic acid into a 100 mL volumetric flask, cooling to room temperature and diluting to volume with dimethyl formamide. Prepare reagent weekly and store in a refrigerator when not in use.		

	(3) Gossypol or Gossypol acetic acid as primary standard. (4) Standard Gossypol solution prepared by weighing 25 mg of primary standard gossypol or 27.9 mg of gossypol acetic acid into a 50 mL volumetric flask. Dissolve in and make up to volume with complexing reagent. Solution is stable for 1 week if stored in refrigerator. The solution contains 0.50 mg gossypol per mL.Multiply gossypol acetic acid with 0.8962 to obtain mg of gossypol.
Method of analysis	1. Grind 50 g sample in a Wiley mill to pass 1 mm sieve. 2. Weigh 0.5 – 0.75 g sample accurately and transfer to a 50 mL volumetric flask. 3. Add 10 mL complexing reagent. 4. Prepare reagent blank containing 10 mL of complexing reagent in a 50 mL volumetric flask. 5. Heat sample and blank in a water bath at 100 °Cfor 30 minutes, cool, dilute to volume with isopropyl alcohol- hexane mixture. 6. Filter through 11 cm filter paper into a 50 mL glass stoppered Erlenmeyer flask discarding first 5 mL of the filtrate. 7. Pipette 2 mL of duplicate sample extract into 25 mL volumetric flasks. 8. Pipette duplicate blank aliquots of same volume as sample aliquot into 25 mL volumetric flasks. 9. Dilute one set of sample and blank aliquots with isopropyl – hexane mixture and reserve as reference solutions for absorption measurement. 10. Add 2 mL of aniline by pipette to the other set of samples and reagent blank aliquots, heat in a water bath for 30 minutes, cool, dilute to volume with isopropyl – hexane mixture and mix well. 11. Allow to stand for 1 h. 12. Measure the absorbance at 440 nm of reagent blank treated with aniline using blank aliquot without aniline as reference solution. 13. Determine absorbance of sample aliquot reacted with aniline using diluted sample aliquot without aniline as reference solution. Subtract absorbance of reagent blank from that of sample aliquot treated with aniline to obtain corrected absorbance. From corrected absorbance of sample aliquot determine mg gossypol in sample aliquot by reference to a calibration graph prepared as in 19.0 (free gossypol).
Calculation	Corrected absorbance for sample = (absorbance of B – absorbance of A) From the corrected absorbance of the sample, determine the mg of gossypol in the sample aliquot by reference to the regression line y=mx+c generated as described above

Reference	AOCS (1989) Official Method Ba 8 – 78
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Titratable Acidity in Tofu		
Method No.	FSSAI 03.036:2022	**Revision No. & Date**	0.0
Scope	This method may be applied for tofu prepared by different methods		
Caution	Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care. Always add pellets to water with cooling.		
Principle	The titratable acidity is expressed as % lactic acid and is determined by titration of a known amount of reconstituted milk with 0.1 N NaOH using phenolphthalein as indicator.		
Apparatus/Instrument	a. Analytical balance ± 0.1 mg b. Burette or Auto-titrator c. Mixer Speed 3800-4000 rpm d. Erlenmeyer flask 100 mL e. 20 mL pipette, other sizes may be used		
Chemicals/Reagents	a. Sodium hydroxide b. Phenolphthalein c. 96% Ethanol		
Preparation of reagents	a. NaOH (0.1 N) standardized with Potassium hydrogen phthalate. b. 1 % Phenolphthalein solution: Dissolve 1g of phenolphthalein in 50 mL 96% ethanol and dilute to 100 mL with deionized water		
Method of analysis	1. Weigh accurately about 2 g of the material in a suitable dish or basin, add 3 mL of hot water and render it to paste; add further 17 mL of hot water washing off any adherents. Cool 2. Add 1 mL of phenolphthalein indicator, shake well and titrate against standard NaOH solution; until a faint pink colour persists for 30 sec. 3. Keep a blank by taking 2 g of material diluted with 20 mL of water in another dish for comparison of colour..		
Calculation	$\%\ titratable\ acidity\ as\ Lactic\ acid = \frac{9 \times A \times N}{W}$ Where: A= volume of standardized NaOH in mL N= Normality of NaOH W= Mass of Tofu		

Reference	IS 1166: 1986 (Reaffirmed year 2018). Specifications for condensed milk, partly skimmed condensed milk (Second Revision). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Acid Value of Extracted Fat		
Method No.	FSSAI 03.037:2022	**Revision No. & Date**	0.0
Scope	The method applies to oil/fat extracted from fried instant noodles and expeller processed flours, soybean and soybean products.		
Caution	Potassium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care. Always add pellets to water with cooling. Petroleum ether: Use only in well ventilated areas. Petroleum ether is extremely flammable. Avoid contact with all ignition sources, including hot surfaces		
Principle	Acid value of oil from fried instant noodles = mg KOH required to neutralize 1 g oil. Oil extracted from noodle is dissolved in alcohol-ether mixture and titrated with alcoholic KOH standard solution.		
Apparatus/Instrument	a. Rotary evaporator b. Water bath c. Air-tight desiccator: silica gel heated at 150 °C is satisfactory drying agent d. Burette (Class A) e. Pipette (Class A).		
Chemicals/Reagents	a. Petroleum ether b. Sodium sulphate c. Potassium hydroxide d. Amidosulfuric acid (certified reference material for volumetric analysis) e. 96% Alcohol f. -Ether g. Phenolphthalein		
Preparation of reagents	a. Alcoholic potassium hydroxide standard solution: 0.05 mol/L: Dissolve 3.5 g potassium hydroxide in equal volume of water (CO2-free) and add ethanol (95%) to 1 L. After mixing, let solution stand for several days keeping the solution CO2-free. Use supernatant after standardization b. Standardization of KOH: Weigh required quantity of amidosulfuric acid (certified reference material for volumetric analysis) and place it into desiccator r (<2.0 kPa) for 48 h. Next, accurately weigh 1 to 1.25 g (recording the weight to 0.1mg), dissolve in water (CO2-free), and		

	dilute to 250 mL Put 25 mL solution into Erlenmeyer flask, add 2 to 3 drops of bromothymol blue indicator and titrate with 0.05 mol/L alcoholic potassium hydroxide solution until colour of solution change to faint blue. c. Calculation: Factor of molarity = (g amidosulfuric acid × purity × 25) / 1.2136 / mL KOH d. Alcohol-ether mixture: equal volumes ethanol (99.5%) and ether. Phenolphthalein solution: Dissolve 1g of phenolphthalein in 50 mL 96% ethanol and dilute to 100 mL with deionized water
Sample preparation	a. Remove instant noodles from package, and leave garnishing and seasoning in package. b. Transfer the noodles to plastic bag to prevent moisture change, and then break these into small fragments with hands or wooden hammer. Select broken noodles in the size range of 2.36 mm to 1.7 mm by using two sieves with 2.36 mm and 1.7 mm openings, and mix well and use for oil extraction. c. If the noodles are too thin to screen with sieves, cut them into 1 to 2 cm lengths, mix well, and use these cut noodles for oil extraction. Extraction of oil a. Weigh 25 g test portion into 200 mL Erlenmeyer flask. b. Add 100 mL petroleum ether to the flask after replacing air in flask by N2 gas. c. Stopper flask and leave for 2 hours. Decant supernatant through filter paper into separating funnel. d. Add 50 mL petroleum ether to residue and filter supernatant through filter paper into the separating funnel. e. Add 75 mL water to the separating funnel and shake well. f. Allow layers to separate and drain the lower aqueous layer. g. Add water, shake, and remove aqueous layer again as done previously. **h.** Decant the petroleum ether layer after dehydration with Na2SO4 into pear-shaped flask. i. Evaporate petroleum ether in the flask on rotary evaporator at not over 40 °C. **j.** Spray N2 gas on extract in the flask to remove all petroleum ether**.**
Method of analysis	1. Before sampling, liquefy extracted oil using water bath. 2. Weigh 1 to 2 g liquefied test portion into Erlenmeyer flask. 3. Add 80 mL alcohol-ether mixture and a few drops of phenolphthalein solution. 4. Titrate with 0.05 mol/L alcoholic KOH until faint pink colour appears and retain for more than 30 s. 5. Perform blank test using only alcohol-ether mixture and

	phenolphthalein solution
Calculation	Acid value $\left[\frac{\text{mg}}{\text{g}}\right] = \frac{(V1 - V0) \times MolarityFactor \times 2.806}{W}$ Where: V1=Titre value for test portion V0= Titre value of blank W=Mass of test portion
Reference	Standard for Instant Noodles CXS 249-2006 Adopted in 2006. Amended in 2016, 2018, 2019.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Fat in Cereals and Cereal-based Products by Randall Extraction- Method		
Method No.	FSSAI 03.038:2022	**Revision No. & Date**	
Scope	Applicable to the analysis of cereal grains and powders (pearl millet/maize flour), solvent extracted flours, expeller pressed flours, textured soy protein, at concentrations from 0.5 to 100% fat. The method is not applicable to baked or expanded products, dried milk or milk products, or oilseeds. It is applicable to the same matrixes as AOAC Official Methods 920.39 and 930.09		
Caution	Store solvents in metal containers in solvent cabinet. Ethers and hexanes are extremely flammable. Have no open flames in the laboratory where the analysis is being performed. Avoid inhaling vapors. Use solvents in a properly operating hood Check each new container of ether for peroxides when it is opened. Also check partial containers of ether that have not been used for several months before using them again. Do not use ether that contains peroxides. Follow manufacturer recommendations for installation, operation, and safety of all extraction equipment. Make sure all solvent is evaporated from cups before placing them in the oven to avoid a fire or explosion.		
Principle	This method is a modification of the standard Soxhlet extraction submerges the test portion in boiling solvent, reducing the time needed for extraction. The solvent dissolves fats, oils, pigments, and other soluble substances, collectively termed "crude fat." A dried, ground test portion is extracted by a 2-step process: In the first step, the thimble containing the test portion is immersed into the boiling solvent. The intermixing of matrix with hot solvent ensures rapid solubilization of extractables. The thimble is then raised above the solvent and the test portion is further extracted by a continuous flow of condensed solvent. The solvent is evaporated and recovered by condensation. The resulting crude fat residue is determined gravimetrically after drying.		
Apparatus/Instrument	a. Solvent extraction system—Multiple position extraction unit conducting 2-stage Randall extraction process with solvent recovery cycle, with Viton or Teflon™ seals compatible with ether or hexanes. b. Thimbles and stand—Cellulose thimbles and stand to hold		

<table>
<tr><td></td><td>thimbles.
c. Extraction cups—Aluminum or glass. (Extraction temperature settings may differ; consult manufacturer's operating instructions.) manufacturers of Randall-type extraction systems.</td></tr>
<tr><td>Chemicals/Reagents</td><td>a. Hexane
b. Anhydrous diethyl ether: Purified for fat extraction. To prevent ether from absorbing water, purchase it in small containers and keep containers tightly closed. Petroleum ether cannot be substituted for diethyl ether because it does not dissolve all of the plant lipid material.114
c. Cotton: Defatted. Soak medical grade cotton in diethyl ether or hexanes for 24 h, agitating several times during this period. Remove and air dry.
d. Sand: ashed (for ignition boats).
e. Celite 545</td></tr>
<tr><td>Sample preparation</td><td>Grind laboratory samples to fineness of 0.75–1 mm.</td></tr>
<tr><td>Method of analysis</td><td>1. Weigh 1–5 g test portions containing ca 100–200 mg fat directly into tared cellulose thimbles, according to following scheme:
<table>
<tr><th>Crude fat (%)</th><th>Test portion weight (g)</th></tr>
<tr><td><2</td><td>5</td></tr>
<tr><td>5</td><td>2–4</td></tr>
<tr><td>10</td><td>1–2</td></tr>
<tr><td>>20</td><td>1</td></tr>
</table>
2. Record weight to nearest 0.1 mg (S) and thimble number.
3. Dry thimbles containing test portions at 102 ± 2 °C for 2 h. If dried test portions will not be extracted immediately, store in desiccator. Both solvent and test materials must be free of moisture to avoid extraction of water-soluble components such as carbohydrates, urea, lactic acid, and glycerol, which will result in false high values.
4. An absorbent, such as diatomaceous earth (Celite or Super-Cel), can be added to the test portion when high fat materials,</td></tr>
</table>

	which melt through the thimble during the pre-dry step, are present. 5. Alternatively, defatted cotton can be added before the pre-dry step to absorb the melted fat. If the material melts at 102 °C, place a pre-tared extraction cup under the thimble during the drying step to catch any melted fat that was unabsorbed and escaped the thimble. Place defatted (with same solvent to be used for extraction) cotton plug on top of test portion to keep material immersed during the boiling step and prevent any loss of test portion from top of thimble. 6. Prepare cotton plug large enough to hold materials in place, yet as small as possible to minimize absorption of solvent. Adding the cotton plug before the 102 ± 2 °C/2 h drying step is acceptable. Place three or four 5 mm glass boiling beads into each cup, and dry cups for at least 30 min at 102 ± 2 °C. Transfer to desiccator and cool to room temperature. 7. Weigh extraction cups and record weight to nearest 0.1 mg (T). 8. Extract, following manufacturer's instructions for operation of extractor. 9. Preheat extractor and turn on condenser cooling water. 10. Attach thimbles containing dried test portions to extraction columns. Put sufficient amount of solvent into each extraction cup to cover test portion when thimbles are in boiling position. 11. Place cups under extraction columns and secure in place. Make sure that cups are matched to their corresponding thimble. 12. Lower thimbles into solvent and boil for 20 min. 13. Verify proper reflux rate which is critical to the complete extraction of fat. This rate depends upon the equipment and should be supplied by the manufacturer. A reflux rate of ca 3–5 drops/s applies to many extraction systems. 14. Raise thimbles out of solvent and extract in this position for 40 min. 15. Then distill as much solvent as possible from cups to reclaim solvent and attain apparent dryness. 16. Remove extraction cups from extractor and place in operating fume hood to finish evaporating solvent at low temperature. (Note: Take care not to pick up any debris on bottom of extraction cup while in hood. Let cups remain in hood until all traces of solvent are gone.) 17. Dry extraction cups in 102 ±2 °C oven for 30 min to remove moisture. Excessive drying may oxidize fat and give high results.

	a. Cool in desiccator to room temperature and weigh to nearest 0.1 mg (F). ***Note: Automated Fat analyser based on Randall Extraction method may also be used in place of the conventional setup following the manufacturer's instructions***
Calculation	$$\%\ Crude\ Fat(hexane\ extract) = \frac{F-T}{S} \times 100$$ $$\%Crude\ fat\ (diethyl\ ether\ extract) = \frac{F-T}{S} \times 100$$ Where: F = weight of cup + fat residue, g; T = weight of empty cup, g; S = test portion weight, g
Reference	AOAC Official Method 2003.06 Crude Fat in Feeds, Cereal Grains, and Forages Randall/Soxtec/Extraction-Submersion Method First Action 2003
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Oil/fat by Soxhlet Extraction		
Method No.	FSSAI 03.039:2022	**Revision No. & Date**	0.0
Scope	Applicable to the analysis of cereal grains and powders (pearl millet/maize flour), soybean and soybean-based products (tofu, soymilk), oilseeds, solvent extracted flours, expeller pressed flours and animal feeds at concentrations from 0.5 to 100% fat. It is applicable to the same matrixes as AOAC Official Methods 920.39 and 930.09		
Caution	Ethers and hexanes are extremely flammable. Have no open flames in the laboratory where the analysis is being performed. Avoid inhaling vapors. Use solvents in a properly operating hood Check each new container of ether for peroxides when it is opened. Also check partial containers of ether that have not been used for several months before using them again. Do not use ether that contains peroxides. Make sure all solvent is evaporated from cups before placing them in the oven to avoid a fire or explosion.		
Principle	The ground sample is extracted with petroleum ether in a Butt-type extraction apparatus such as Soxhlet distiller or similar devices. The solvent is distilled off and the residue dried and weighed.		
Apparatus/Instrument	a. Soxhlet extraction apparatus b. Extraction thimbles, free of matter soluble in petroleum ether and having porosity consistent with the requirements c. Water bath or steam bath d. Extraction cups/thimbles e. Analytical balance (Accuracy 0.01 g).		
Chemicals/Reagents	Anhydrous Petroleum ether, boiling range: 40 to 60 ℃.		
Sample preparation	1. Grind laboratory samples to fineness of 0.75–1 mm 2. Dry the sample at 102± 2 °C and determine the moisture content. 3. Note: If dried test portions are not extracted immediately, store in desiccator. Both solvent and test materials must be free of moisture to avoid extraction of water-soluble components such as carbohydrates, urea, lactic acid, and glycerol, which will result in false high values.		

Method of analysis	1. Weigh 2 g of moisture free ground sample and enclose the sample in filter paper. 2. Place the sample in the Butt tube device 3. Turn on the heating mantle and extract the sample with petroleum ether for 4-6 H at condensation rate of 5 to 6 drops per second. 4. Evaporate the petroleum ether on a steam bath or in a water bath. 5. Weigh the mass of the extracted oil. **Note:** To get accurate and reliable results, it is important that the powder sample is fine enough as it has been found that particle size of the ground soybean affects the extraction. Place the thimble in extraction cup then place the cup in extractor and pour 150 mL of petroleum ether.
Calculation	$$\%\ oil\ (moisture\ free\ basis) = \frac{W2}{W1} \times 100$$ Where: W1= Mass of sample W2=Mass of oil
Reference	AOAC 948.22 21st Edn. (2019). Fat (Crude) in Nuts and Nut Products. AOAC International, USA. AOAC Official Method 920.39 Fat (Crude) or Ether Extract in Animal Feed
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Urease Index		
Method No.	FSSAI 03.040:2022	**Revision No. & Date**	0.0
Scope	Applicable to soybean meals, soy flour and other soybean products		
Principle	The method determines the residual urease activity of soybean products as an indirect indicator to assess whether the anti-nutritional factors, such as trypsin inhibitors, present in soybeans have been destroyed by heat processing. The test measures the increase in pH consequence of the release of ammonia, which is alkaline, into the media arising from the breakdown of urea by the urease present in soybean products (urea is broken down into ammonia and carbon dioxide).		
Apparatus/Instrument	a. Analytical balance (sensitivity 0.01 g) b. pH meter: Calibrate with standard buffer pH 7.0 and 9.) prior to use c. Shaking water bath set at 30 ±2 °C		
Chemicals/Reagents	a. Urea b. Disodium Hydrogen phosphate (Na_2HPO_4) c. Potassium Dihydrogen phosphate (KH_2PO_4)		
Preparation of reagents	Buffered urea: Dissolve 30 g of urea into 1 L of a buffer solution, composed of 4.45 g of Na2HPO4 and 3.4 g of KH2PO4 (pH 7.0)		
Sample preparation	Grind the sample to a fine powder		
Method of analysis	1. Weigh approximately 200 mg of the processed legumes powder 2. Add 10 mL of buffered urea 3. Prepare blank containing buffered urea. 4. Incubate test and blank in a water bath set at 30 °C for 30 min. 5. The tubes were agitated at 5 min intervals. 6. Measure pH exactly 5 min after removal from water bath. 7. Determine pH and compare it with the original pH of the urea solution. 8. The difference between the pH of test and blank is the indicator of urease activity 9. Each assay is carried out in triplicate.		
Calculation/Interpretation	Urease index= pH of sample-pH of blank		

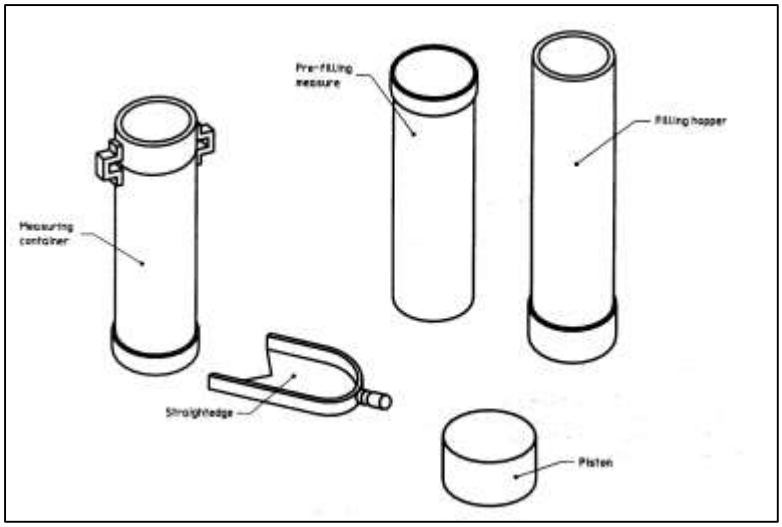
	Urease index values of 0.05 to 0.2 pH rise are considered for properly processed soybean meal. Values above 0.2 indicated under-heating and values below 0.05 indicated over-heating.
Reference	Urease Activity. Official Method Ba 9-58. Official Methods and recommended Practices of the AOCS, AOCS, 6th ed., Second Printing, Urbana, IL
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Test weight: One Litre Mass		
Method No.	FSSAI 03.041:2022	**Revision No. & Date**	0.0
Scope	Test weight is the weight of a measured volume (1 L) of grain expressed in g/L. The method is applicable to all cereals and grains.		
Principle	Test weight is used as an indicator of general grain quality and is a measure of grain bulk density and grain-soundness. The mass of 0.5 L of grain is measured.		
Apparatus/Instruments	a. 0.5 litre measure: A cylindrical shaped cup with an inside diameter of approximately 90 mm and a height of approximately 77.5 mm. The measure is calibrated to contain 500 mL of water, ± 1 mL, at 20 °C. b. Cox funnel: A funnel with a 3.81 cm opening and a drop of 4.41 cm, from the opening in the funnel to the top of the measure used to uniformly direct the flow of grain into the 0.5 litre cup. c. Striker: A piece of round hardwood, 2.2 cm in diameter and approximately 23 cm in length. d. Analytical balance (Sensitivity 0.1 g)		
Materials and Reagents	Cleaned seed: ~1 kg		
Method of analysis	1. Fill the 0.5 litre measure to overflowing with the grain to be tested. 2. Ensure the slide is inserted into the Cox funnel. 3. Pour the contents of the 0.5 litre measure, plus an extra handful, into the Cox funnel. 4. Place the 0.5 litre measure on a solid base. 5. Position the Cox funnel on top of the 0.5 litre measure so that the notched legs of the Cox funnel fit securely onto the measure's rim. 6. Remove the slide on the Cox funnel quickly so that the grain drops evenly into the 0.5 litre measure. 7. Carefully remove the Cox funnel from the top of the 0.5 litre measure so as not to disturb the grain. Any jarring or tapping of the cup at this point will result in compaction of the grain in the 0.5 litre measure and could produce inaccurate results. 8. Place the hardwood striker on the rim of the 0.5 litre measure and, using three zigzag, equal motions, scalp off the excess the grain in the measure.		

	9. Pour the grain remaining in the 0.5 litre measure into the scale pan. 10. Determine the weight in grams of the grain in the scale pan. 11. Calculate the mean of the three. 12. Convert the grams in the 0.5 litre measure to g/L
Calculation with units of expression	$1 \text{ Litre mass (in gms)} = 0.5\ L\ mass \times 2$
Reference	Determining test weight: Official grain grading guide. Canadian Grain Commission. 2021 https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/oggg-aug-1-2021-en.pdf
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Thousand Seed/Kernel Weight (TSW)		
Method No.	FSSAI 03.042:2022	**Revision No. & Date**	0.0
Scope	The method for TSW is applicable to all cereals and grains listed in Food Safety and Standards Rules and Regulations 2011.		
Principle	Pure seeds are counted and weighed by one of two methods: 1. counting the whole pure seed fraction, 2. counting replicates of 100 seeds. The fraction or replicates with known numbers of pure seeds are then weighed and the TSW determined. Seeds are counted: – using either a counting machine or – manually (or – using a counting tool, e.g. counting boards		
Apparatus/Instruments	a. Counting machine b. Counting board c. Analytical balance (sensitivity0.1 g)		
Materials and Reagents	None		
Method of analysis	Method 1: Counting the whole seed fraction 1. The whole seed fraction is counted using a counting machine: 2. Put the whole seed fraction through a) the counting machine, and read the number of seeds on the indicator or b) Manual counting: Count the seeds in the whole seed fraction and record the number of seeds. 3. In either case (a and b), weigh the counted seed fraction in grams. Method 2: Counting replicates 1. Count out at random, by hand or with a counting tool, eight replicates, each of 100 pure seeds. 2. Weigh each replicate in grams 3. Calculate the variance, standard deviation and coefficient of variation 4. If the coefficient of variation does not exceed 4.0 the result of the mean determination can be used for further calculation.		
Calculation with units of expression	Method 1: Calculate the weight of 1000 seeds from the weight of the whole pure seed fraction. $$\text{Weight of 1000 seeds} = \frac{\text{Sample weight}}{\text{Number of seeds counted}} \times 1000$$ Method 2: Calculate the average weight of 1000 seeds from the weights of eight or more 100-seed replicates. $$\text{Weight of 1000 seeds} = \frac{\sum \text{Weight of 100 seed replicates}}{\text{Number of 100 seed replicates}} \times 10$$		

Reference	Chapter 10: Thousand-seed weight (TSW) determination. International Rules for Seed Testing 2019 The International Seed Testing Association (ISTA)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Bulk Density (Mass per Hectoliter) of Cereals		
Method No.	FSSAI 03.043:2022	**Revision No. & Date**	0.0
Scope	The method specifies a routine method for the determination of bulk density, called "mass per hectolitre", of cereals (wheat, barley, oats and rye), utilizing a One Litre measuring container.		
Caution	The bulk density as described should not be confused with the "packing density" or the intrinsic density of the cereals.		
Principle	It involves the principal of weighing a known volume of grain and determining its bulk density in kilograms per hectoliter (Kg/hL). The grain sample is dropped down a stainless steel chondrometer under the restriction of a falling weight. The sample is weighed and converted to Kg/hl by use of a conversion chart.		
Apparatus/Instruments	a) A chondrometer comprising of: Pre-filling measure: The pre-filling measure shall be made of metal and be in the shape of a straight-sided cylinder, closed at the bottom end with a flat base plate. On its internal wall there shall be an annular level mark, placed no less than 1 cm and no more than 3 cm from the open end of the cylinder. Dimensions: *Note*: The purpose of the pre-filling measure is to control the manner in which the filling hopper is filled with grain and thus to reduce or eliminate operator errors which might otherwise arise.  Apparatus for determining the bulk density of cereals utilizing a 1 Litre measuring container (Chondrometer). Adapted from *IS* Method of Analysis for food-grains. Part 3: Determination of Hectolitre Weight (*First Revision)* IS 4333 (Part 3) :2002 ISO7971-2:1999 Reaffirmed – 2012 Filling hopper: The hopper shall be made of metal and be in the shape of a straight-sided cylinder, open at both ends. At the bottom of the cylinder an		

	extended projection around the circumference of the cylinder enables the filling hopper to be pushed onto the measuring ring at the top of the measuring container The hopper receives from the pre-filling measure a volume of grain greater than one Litre. Measuring container with measuring ring: The 1 Litre volume of the measuring container is formed by the internal surface of the container wall, the upper surface of the inserted piston and the lower surface of the fully inserted straightedge. The maximum permissible relative error on the capacity of the container is ±3/1 000. The wall of the measuring container shall be made of a seamless drawn-brass tube or a stainless-steel tube in the shape of a straight-sided cylinder, open at the top and closed at the base, and shall have an external reinforcement on the edge. The measuring ~edge shall be ground flat. *A measuring ring:*the internal diameter of which is the same as that of the measuring container, shall be attached to the measuring container over the measuring edge. The gap between the measuring edge and the measuring ring shall be large enough for the straight tedge to be able to be pushed through easily but without any noticeable clearance. The base of the measuring container shall be flat and perforated so as to allow the escape of air during use of the apparatus. The external reinforcement encircling the base of the measuring container and its three feet shall be in one piece. It shall be soldered to the measuring container wall and be secure against shifting. *Piston*: The piston shall be made of brass plate in the shape of a straight-sided cylinder with flat ends. Internally, shall be stiffened such that the stamping (see clause 10) may be carried out without the surface being dented. If the piston should be dented, or otherwise damaged, it shall be replaced because the dent would alter the volume of the grain being tested. When the straightedge is withdrawn, the piston falls smoothly down the measuring container, thus driving air through the exit holes in the base of the measuring container. This therefore controls the rate of fall and ensures the smooth flow of grain from the filling hopper into the measuring container. *Straightedge (levelling blade)*: The straightedge shall be a flat, thin but rigid, hardened-steel blade, equipped with a handle. The surfaces shall be flat and parallel. It shall be large enough to cover the cross-section of the measuring container completely at its limit of travel. The blade shall be cut to the form of an open V at the front, and bevelled such that the line of cutting is in the middle of the thickness of the blade. The blade slides horizontally into the slot in the measuring container and is pushed manually through the grain, guided by the slot, in a smooth and continuous movement. This separates precisely 1 Litre of grain (below the blade) from excess grain above the blade. *Base plate*: The base plate shall be made of metal and arranged such that the measuring container can be firmly connected to it by simply rotating. It shall not be perforated. It shall be fixed to a mounting plate of hardwood or to the hardwood lid of the transport case for the apparatus. The mounting plate or the

<table>
<tr><td></td><td colspan="3">transposition case shall be provided with vertical-adjustment screws and a spirit level such that, when placed on a flat horizontal surface, the apparatus stands firm and vertical, otherwise errors will be introduced.
b) Analytical balance (readability 0.01 g)</td></tr>
<tr><td>Dimensions of different parts of the apparatus</td><td colspan="3"><table>
<tr><td rowspan="2">Pre-filling measure</td><td>Capacity to level mark</td><td>1.350± 10 ml</td></tr>
<tr><td>Internal diameter</td><td>86 ± 0 .2 mm</td></tr>
<tr><td rowspan="3">Filling hopper</td><td>Internal diameter</td><td>79 ± 0 .1 mm</td></tr>
<tr><td>Wall thickness</td><td>1 ± 0 .2mm</td></tr>
<tr><td>Height above piston</td><td>280 ±2mm</td></tr>
<tr><td rowspan="3">Piston</td><td>Diameter</td><td>87 .5 ±0 .1mm</td></tr>
<tr><td>Height</td><td>40 ± 0 .2mm</td></tr>
<tr><td>Mass</td><td>450 ±2g</td></tr>
<tr><td rowspan="11">Measuring container</td><td>Internal diameter</td><td>88 .2 ± 0 .1mm</td></tr>
<tr><td>Internal height above piston</td><td>163 .7 ± 0 .1mm</td></tr>
<tr><td>Wall thickness</td><td>1 .2 ±0 .5mm</td></tr>
<tr><td colspan="2">External reinforcement of upper edge</td></tr>
<tr><td>Thickness
height</td><td>2 .5 ±0 .5mm
6 .0 ±1 .0mm</td></tr>
<tr><td>Base thickness</td><td>4 .5 ± 0 .1mm</td></tr>
<tr><td>Diameter of base perforations</td><td>3 .0 ±0 .1mm</td></tr>
<tr><td>Height of feet</td><td>9 .0 ±0 .1mm</td></tr>
<tr><td>Diameter of feet</td><td>6 .0 ± 0 .1mm</td></tr>
<tr><td>Gap between base and base plate</td><td>6 .0 ±0 .1mm</td></tr>
<tr><td>Number of perforations in base</td><td>1+4+8+12+16+20+24=85</td></tr>
<tr><td rowspan="2">Measuring ring:</td><td>internal diameter</td><td>88 .2 ±0 .1mm</td></tr>
<tr><td>height</td><td>40 .5 ±0 .1 mm</td></tr>
<tr><td>Baseplate</td><td>Diameter of locating circle</td><td>80 .0 ± 0 .1 mm</td></tr>
<tr><td rowspan="3">Straightedge</td><td>Thickness</td><td>1 ±0 .05 mm</td></tr>
<tr><td>Cut-out angle</td><td>90°±2°</td></tr>
<tr><td>Width of bevel of cutting edge</td><td>3 ± 0 .5 mm</td></tr>
</table></td></tr>
<tr><td>Sample Preparation</td><td colspan="3">The grain sample shall be air-dried, free from foreign bodies and have achieved ambient temperature. The atmospheric relative humidity of the room shall be between 40 % and 75 %.
Note: It is recommended to determine the moisture content of the grain in</td></tr>
<tr><td>Method of analysis</td><td colspan="3">1. Install the apparatus vertically and free from vibrations on a firm, non-sprung base.</td></tr>
</table>

	2. Before each filling, ensure that the measuring container, slit and piston are free from dust and grain residues or other foreign bodies. 3. Fix the measuring container to the base plate and push the straightedge into the slit of the measuring container in such a way that the inscription "Top" can be seen from above. 4. Place the piston on the straightedge in such a way that the surface bearing the production number is uppermost. 5. Put on the filling hopper in such a way that its production number can be seen from the front. 6. Fill the pre-filling measure with the sample of grain up to the level mark. 7. Then empty it to within 3 cm or4 cm from the upper edge of the filling hopper in such a way that the grain sample flows evenly into the middle of the filling hopper in 11 s to 13 s. 8. After filling, quickly pull out the straightedge, but without shaking the apparatus. 9. When the piston and the grain have fallen into the measuring container, place the straightedge back in the slit and push it through the grain in a single stroke. 10. If a particle becomes jammed between the slit edge and the straightedge in the process, the pouring shall be repeated. 11. Throw out excess grain lying on the straightedge. 12. Then remove the filling hopper and straight edge. 13. Throughout the procedure it is important that the apparatus should not be tapped, knocked or shaken, otherwise a falsely high result will be obtained. 14. However, once the 1 litre volume has been isolated, this restriction need not be observed. 15. Weigh the contents of the measuring container to the nearest 1 g using the weighing device 16. Alternatively, the grain may be poured into a separate previously tared receptacle and weighed to the nearest1 g.
Calculation with units of expression	To determine the bulk density, expressed in kilograms per hectoliter, take the mass in grams of the cereal contained in the 1 Litre measuring container (m) and apply the following equation. Bulk density, in kilograms per hectolitre, equals Wheat (kg/hL) = 0.1002 m+0.53 Barley(kg/hL) = 0.1036 m–2.22 Rye (kg/hL) = 0. 1017m–0.08 Oats (kg/hL) = 0.1013m–0.61 Express the result to the nearest 0.1 kg/hi at a stated moisture content. Note: The equations provide linear mathematical conversions from grams per litre to kilograms per hectolitre.

	The factors are derived from *Tables of the determination of mass per hectolitre of wheat, barley, rye and oats.(Brunswick: Physikalisch-Technische Bundesanstalt, 1967).*
Reference	ISO 7971-3:2019(en) , Cereals — Determination of bulk density, called mass per hectolitre — Part 3
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Solids in Soy Beverage		
Method No.	FSSAI 03.044:2022	**Revision No. & Date**	0.0
Scope	This method is applicable to soymilk		
Caution	Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature.		
Principle	The sample is dried over a water bath and then in an oven to remove all moisture and remaining solids weighed.		
Apparatus/Instruments	a. Analytical balance: Readability 0.0001g b. Moisture dish c. Water bath or hotplate d. Hot air oven		
Sample Preparation	Homogenize the soymilk		
Method of analysis	1. Pipette 2 mL of homogenous liquid in a previously dried and weighed aluminum dish provided with a tight-fitting lid and weigh. 2. Remove the lid on the dish and place on a water bath till the sample is dry. 3. Keep the dish in air oven at 98±2 °C for 90 min, 4. Cool in a desiccator and weigh. 5. Repeat heating and weighing till constant weight is obtained (within 2 mg).		
Calculation with units of expression	$$Total\ solids(\%) = \frac{W3 - W1}{W2} \times 100$$ Where: W1 = initial weight of empty moisture dish W2 = weight of soymilk W3 = final weight of moisture dish with dried sample		
Reference	IS 12333 - 2017/ ISO 6731: 2010. Milk, Cream and Evaporated milk. – Determination of total Solids Content -reference method. Bureau of Indian Standards, News Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Moisture in Edible Starches and Starch-Products		
Method No.	FSSAI 03.045:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to edible starches (tapioca and Palm) and starch products like Sago (Saboodana)		
Caution	Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care. Stand to one side when opening the door to avoid high temperature		
Principle	The method is based on the dehydration of the test portion in an electrically heated drying oven at a temperature of 130 to 133 °C atmospheric pressure for a period of 1 hour 30 minutes.		
Apparatus/Instruments	a. Analytical balance: Sensitivity 0.001g b. Metal dish: Unaffected by starch under the conditions of test, for example, aluminium, and having a suitable tight-fitting lid, suitable dimensions are 55 to 65 mm diameter, 15 to 30 mm height and a bout O-5 mm wall thickness. c. Hot air oven Constant-temperature, electrically heated and with a suitable air circulation.		
Sample Preparation	For pure starches, take the starch powder as required for different tests. For starch products, take about 100 g of the material and grind it coarsely in a pestle and mortar pestle and mortar so that the whole of it passes through 250-micron IS Sieve. Place this prepared material in a clean and dry stoppered glass bottle.		
Method of analysis	1. Weigh the dish and its lid after drying at 130 °C and cooling in the desiccator. 2. Transfer 5 -25 g of the well-mixed sample, which shall be free from any hard and lumpy material, to the dish with the minimum exposure to the atmosphere. 3. Replace the lid and weigh immediately to determine the mass of the test sample. 4. Distribute the test portion in a uniform layer over the bottom of the dish. 5. Place the open dish containing the test sample in the drying oven preheated to 130 °C, allowing the lid to lean against the dish, and dry at 130 to 133 °C for 1 hour		

	30 minutes reckoned from the moment when, the oven temperature again reaches 130 °C. 6. After this period, rapidly cover the dish and put it in the desiccator. 7. The dishes should never be superimposed in the desiccator. 8. Allow the test sample to cool to room temperature in the desiccator for 30 to 45 minutes. 9. When the dish has cooled to room temperature, weigh it within 2 minutes of its removal from the desiccator. 10. Carry out at least two determinations on the same well-mixed laboratory sample. *Note: The difference between the results of two determinations, carried out simultaneously or in rapid succession by the same analyst, shall not exceed 0.2 g in 100 g of the product.*
Calculation with units of expression	$$Moisture\ (\%\ m/m) = \frac{W3 - W1}{W2 - W1} \times 100$$ Where: W1 = initial mass of empty moisture dish in g W2 = Mass of moisture dish with sample W3 = Mass weight of moisture dish with dried sample Note*: Take as the result the arithmetic mean of the two determinations, if the requirements concerning repeatability are satisfied. Report the result to the first decimal place.*
Reference	IS: 4706 (Part II) – 1978 (Reaffirmed 2005) Indian Standard methods of test for edible starches and starch products Part ii Chemical methods.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Starch Content: Acid Hydrolysis Method		
Method No.	FSSAI 03.046:2022	**Revision No. & Date**	0.0
Scope	The method based on the use of acid and is applicable to the determination of total starch in cereals, flours, edible starches, sago and complex media, including all starchy products (food and feed, digestive contents) and glycogen		
Caution	Concentrated hydrochloric acid Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care. Hold the titration flask with insulated gloves to avoid burns. The titration should be completed in 3 minutes ± 5 seconds from the commencement of boiling. The heating device used for boiling the reaction mixture during the titration is of prime importance when accurate results are to be guaranteed. During the whole time, the flask should remain on the wire gauze and boil at a moderate rate. The continuous emission of steam from the neck prevents atmospheric oxidation of the Fehling's solution or of the indicator. During the additions of sugar solution to the boiling liquid, the main burette tube must be kept out of the steam outlet while the jet is brought over the mouth of the flask		
Principle	Acid hydrolysis is used to convert starch into dextrose. The Lane-Eynon titration method is used to determine the concentration of dextrose in the hydrolysate. A burette is used to add the carbohydrate solution being analyzed to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The reducing sugars in the solution react with the copper sulfate present in the flask. Once all the copper sulfate in solution has reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point is recorded.		
Apparatus/Instruments	a. Analytical balance: (Readability 0.0001 g) b. Burette Class A- 50 mL c. Conical Flask		
Materials and Reagents	a. Concentrated hydrochloric acid (sp gr 1.16) b. Sodium carbonate c. Benzoic acid d. Ethyl Ether e. Ethyl Alcohol f. Methylene Blue		

	g. Copper sulphate ($CuSO_4$. $5H_2O$) h. Rochelle salt (Potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)) i. Concentrated sulphuric acid (sp gr 1.84) j. Sodium hydroxide k. Anhydrous D-glucose-Dry two hours at 100 °C and cool in desiccator before use.
Preparation of Reagents	a. Ethyl Alcohol - 10 percent (v/v). b. Dilute Hydrochloric Acid: 2.5% prepared by mixing 20 mLof and 200 mL of water. c. Sodium carbonate solution 20% (m/v): Weigh 20 g of sodium carbonate and dissolve in water to a final volume of 100 mL d. Stock Solution of Dextrose: Weigh accurately 10 g of anhydrous dextrose into a one-litre graduated flask and dissolve it in water. Add to this solution 2.5 g of benzoic acid, shake to dissolve benzoic acid and make up the volume to the mark with water. After 48 hours this solution should not be used e. Standard Dextrose Solution: Dilute a known aliquot of the above stock solution with water to such a concentration that more than 15 mL but less than 50 mL of it will be required to reduce all the copper in the Fehling's solution taken for titration. *Note the concentration of anhydrous dextrose in this solution as mg/100mL Prepare this solution fresh every day.* NOTE - When 10 mL of Fehling's solution are taken for titration, a standard dextrose solution containing O-11 to 0.30 percent (M/V) of anhydrous dextrose is convenient for use e. Methylene Blue indicator solution - Dissolve 0.2 g of methylene blue in water and dilute to 100 mL. f. Fehling's Solution (Soxhlet Modification): Prepared by mixing immediately before use, equal volume of solution A and solution B which are prepared as follows: a*) Solution A* -Dissolve 34.639 g of copper sulphate ($CuSO_4$. $5H_2O$) in water, add O-5 mL of concentrated sulphuric and dilute to 500 mL in a graduated flask. Filter the solution through prepared asbestos. b) *Solution B* - Dissolve 173 g of Rochelle salt and 50 g of sodium hydroxide in water, dilute to 500 mL in a graduated flask and allow the solution to stand for two days. Filter this solution through prepared asbestos. c) *Standardization of Fehling's Solution* - Pour the standard dextrose solution into a 50-ml burette. Find the titre (that is, the volume of the standard dextrose solution required to reduce all the

copper in 10 ml of Fehling's solution) corresponding to the concentration of the standard dextrose solution from Table 1. (If, for example, the standard dextrose solution contains 167.0 mg of anhydrous dextrose per 100 ml, the corresponding titre would be 30 ml.) Pipette10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the standard dextrose solution required to effect reduction of all the copper, so that not more than 1 ml will be required later to complete the titration. Heat the flask containing the mixture over a wire gauze. Gently boil the contents of the flask for two minutes. At the end of two minutes of boiling, add, without interrupting boiling, one ml of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add standard dextrose solution (one or two drops at a time) from the burette till the blue colour of the indicator

just disappears. [The titration should be completed within one minute, so that the contents of the flask boil altogether for three minutes without interruption.

Note: In adding sugar solution to the reaction mixture, the burette may be held in hand over the flask. The burette may be fitted with a small outlet tube bent twice at right angles, so that the body of the burette may be kept out of the steam while adding the sugar solution. Burettes with glass taps are unsuitable for this work, as the taps become heated by the steam and are liable to jam.

Note the titre. multiply the titre (obtained by direct titration) by the number of milligrams of anhydrous dextrose in 1 ml of the standard dextrose solution to obtain the dextrose factor. Compare this factor with dextrose factor given in Table below.

Determine the correction, if any, to be applied to the dextrose factors derived from Table below.

Example:

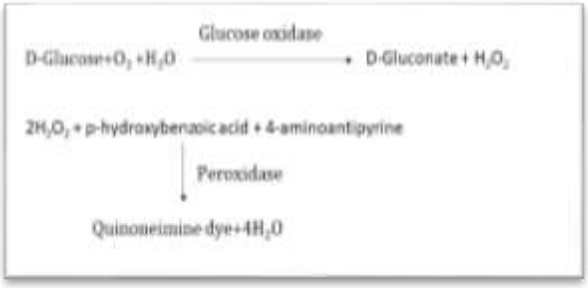
Concentration of anhydrous dextrose in the standard dextrose solution as mg/100mL	= 167.0
Titre obtained by direct titration	= 30.1 m
Dextrose factor for 30.1 ml of the standard dextrose solution	=titre in ml × number of mg of anhydrous dextrose in 1 ml of the standard dextrose solution = 30.1 x 1.670 = 50.2670

	Dextrose factor for 30.1 ml of standard dextrose solution from Table calculated by interpolation) = 50.11 Correction to be applied to the dextrose factors derived from Table below = 50.2670 - 50.11 = + 0.1570
Sample Preparation	Mill approx. 50 g of sample (cereal, sago or food product) to pass a 0.5 mm sieve using a centrifugal mill. Homogenize sample by shaking and inversion. Determined moisture content by recording weight loss on storage of flour samples (0.5 g) at 103 ºC for 16 h or until weight stabilization. Use information on final calculation
Method of analysis	***Preparation of the solution (Starch hydrolysis)*** 1. Extract about 0.5 g of ground material, accurately weighed, with five 10-ml portions of ether on a filter paper that would retain completely the smallest starch granules. Evaporate the ether from the residue and wash with 150 ml of 10% ethyl alcohol. 2. Carefully wash off the residue from the filter paper with 200 ml of cold water. 3. Heat the undissolved residue with 220 ml of 2.5 percent dilute hydrochloric acid in a flask equipped with reflux condenser for 24 hours. 4. Cool and neutralize with sodium carbonate solution and transfer quantitatively to a250-ml graduated flask and make up to volume. ***Incremental Method of Titration*** 1. Pour the prepared hydrolysate into a 50-mLburette (the same may be filtered if not clear 2. Pipette 10 ml of Fehling's solution into a 300 ml conical flask and run in from the burette 15 ml of the prepared solution. 3. Without further dilution, heat the contents of the flask over a wire gauze, and boil. (After the liquid has been boiling for about 15 seconds, it will be possible to judge if almost all the copper is reduced by the bright red color imparted to the boiling liquid by the suspended cuprous oxide). 4. When it is judged that nearly all the copper is reduced, add 1 ml of the methylene blue indicator solution. 5. Continue boiling the contents of the flask for one to two minutes from the commencement of boiling, and then add the prepared solution in small quantities (1 ml or less at a time), allowing the liquid to boil for about 10 seconds between successive additions, till the blue colour of the indicator just disappears 6. In case there still appears to be much unreduced copper after the

	mixture of Fehling's solution with 15 ml of the prepared solution has been boiling for 15 seconds, add the prepared solution from the burette in larger increments (more than 1 ml at a time, according to judgement), and allow the mixture to boil for 15 seconds after each addition. 7. Repeat the addition of the prepared solution at intervals of 15 seconds until it is considered unsafe to add a large, increment of the prepared test solution. 8. At this stage continue the boiling for an additional one to two minutes, add 1 ml of methylene blue indicator solution and complete the titration by adding the prepared solution in small quantities (less than 1 ml at time). *NOTE 1 -It is advisable not to add the indicator until the end point has been nearly reached because the indicator retains its full colour until the end point is almost reached and thus gives no warning to the operator to go slowly*. *NOTE 2 - When the operator has had a fair amount of experience with the method, a sufficiently accurate result may often be obtained by a single estimation by the incremental method of titration. For the utmost degree of accuracy of which the method is capable a second titration should be carried out by the standard method of titration*. *9.* Repeat titration twice and calculate the mean of three parallel titrations ***Standard method of titration*** 1. Pipette 10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the prepared solution required to effect reduction of all the copper so that, if possible, not more than one ml will be required later to complete the titration. 2. Gently boil the contents of the flask for two minutes. 3. At the end of 2 minutes of boiling, add without interrupting the boiling, one ml of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add the prepared solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears (see Note 1). 4. The titration should be completed within one minute, so that the contents of the flask boil altogether for 3 minutes without interruption (see Note 2).] Note:1 The indicator is so sensitive that it is possible to determine the end point within one drop of the prepared test solution in many cases. The complete decolourization of the methylene blue is usually indicated by the whole reaction liquid in which the cuprous oxide is continuously churned up becoming bright red or orange in colour. In

<table>
<tr><td></td><td>case of doubt, the flame may be removed from the wire gauze or one or two seconds and the flask held against a sheet of white paper. The top edge of the liquid would appear bluish if the indicator is not completely decolourized. It is inadvisable to interrupt the boiling as the indicator undergoes back oxidation rather rapidly when air is allowed free access into the flask, but there is no danger of this as long as a continuous stream of steam is issuing from the mouth of the flask.
Note 2 -It should be observed that with both incremental and standard methods of titration, the flask containing the reaction mixture is left on the wire gauze over the flame throughout the titration.
NOTE 4 - The dilution of the test solution (starch hydrolysate) should be such that its titre value lies between15 and 50 ml around 25 ml.</td></tr>
<tr><td>Calculation with units of expression</td><td>10 mL Fehling solution contains 0.11 gm cupric oxide which is able to oxidize 0.05 gm of dextrose (glucose)
Calculate the dextrose content of the prepared solution as follows:
$$\text{m} = \frac{\text{Dextrose factor}}{\text{Titre}}$$
$$\text{Starch (on dry basis), \% mass} = \frac{9.3 \times m \times V}{M1(100 - M)}$$
Where:
m=Milligrams of anhydrous dextrose present in 1 ml of the prepared solution
V=total volume in ml of the prepared solution
M1= mass in g of the material used to prepare V ml of the solution
M= percentage of moisture
<table><tr><td colspan="4">Dextrose Factors for 10 mL of Fehling’s solution</td></tr><tr><td>Titre value</td><td>Dextrose factor*</td><td>Titre value</td><td>Dextrose factor*</td></tr><tr><td>15</td><td>49.1</td><td>33</td><td>50.3</td></tr><tr><td>16</td><td>49.2</td><td>34</td><td>50.3</td></tr><tr><td>17</td><td>49.3</td><td>35</td><td>50.4</td></tr><tr><td>18</td><td>49.3</td><td>36</td><td>50.4</td></tr><tr><td>19</td><td>49.4</td><td>37</td><td>50.5</td></tr><tr><td>20</td><td>49.5</td><td>38</td><td>50.5</td></tr><tr><td>21</td><td>49.5</td><td>39</td><td>50.6</td></tr><tr><td>22</td><td>49.6</td><td>40</td><td>50.6</td></tr></table></td></tr>
</table>

	23	49.7	41	50.7
	24	49.8	42	50.7
	25	49.8	43	50.8
	26	49.9	44	50.8
	27	49.9	45	50.9
	28	50.0	46	50.9
	29	50.0	47	51.0
	30	50.1	48	51.0
	32	50.2	49	51.0
	32	50.2	50	51.1
	*Milligrams of anhydrous dextrose corresponding to 10 mL of Fehling's solution			
Reference	IS: 4706 (Part II) – 1978 (Reaffirmed 2005) Indian Standard methods of test for edible starches and starch products Part ii Chemical methods.			
Approved by	Scientific Panel on Methods of Sampling and Analysis			

	Determination of Total Starch Content: Enzymatic Method		
Method No.	FSSAI 03.047:2022	**Revision No. & Date**	0.0
Scope	The method based on the use of thermostable α-amylase and amyloglucosidase, is applicable to the determination of total starch in cereals, flours, edible starches, sago and complex media, including all starchy products (food and feed, digestive contents) and glycogen		
Caution	*Sodium azide*: Glucose oxidase/peroxidase-aminoantipyrine buffer mixture, MOPS, and acetate buffers contain sodium azide. Avoid con tact with skin and eyes. In case of contact, immediately flush contact surfaces with plenty of water. Disposal of these re agents into sinks with copper or lead plumbing should be followed immediately with large quantities of water to pre vent potential explosive hazards. *Dimethyl sulfoxide* is a skin irritant and should be used with caution		
Principle	Starch is measured as glucose by an enzymatic colorimetric assay, after initial gelatinization in an autoclave, followed by enzymatic hydrolysis Thermostable α-amylase hydrolyses starch into soluble branched and unbranched maltodextrins. $\text{Starch} + H2O \xrightarrow{\alpha\text{-amylase, pH 5.0 or 7.0, 100°C}} \text{Maltodextrins}$ Amyloglucosidase (AMG) quantitatively hydrolyses maltodextrins to D-glucose. $\text{Maltodextrins} + H_2O \xrightarrow{\text{Amyloglucosidase}} \text{D-Glucose}$ Resulting D-glucose is determined directly using the Glucose oxidase-peroxidase (GODPOD) reagent by conversion to a red-coloured quinoneimine dye compound through the combined action of glucose oxidase and peroxidase. The assay is specific for α-glucans (including starch, glycogen, phytoglycogen and non-resistant maltodextrins). 	

Apparatus/Instruments	a. Grinding mill and 0.5 mm sieve, or similar device. b. Benchtop centrifuge: capable of holding 101 x 65 mm c. polypropylene tubes, with rating of approx. 3,250 × g(~ 4,000 rpm), d. UV-Vis Spectrophotometer e. Analytical balance: 0.0001 g readability, accuracy and precision. f. Thermostatted water bath: Maintaining 50° ± 0.1°C g. Boiling water bath: with tube rack —Boiling water at 95°–100°C. h. Magnetic stirrer i. Vortex mixer j. pH Meter. k. Stop clock timer (digital). l. Oven with forced-convection; maintaining 103° ± 1°C; used for determining dry weight of test sample m. Micropipettes: capable of delivering 100 µL or 1.0 mL, e.g. with disposable tips. n. Positive displacement pipettes o. Dispensers p. Disposable polypropylene tube.— 13 mL, 101 x 16.5 mm q. Disposable 2.0 mL polypropylene microfuge tubes.— r. Glass test tubes: 16 x 100 mm, 14 mL capacity. s. Digestion tubes: Culture tubes (16 x 120 mm with screw caps.
Materials and Reagents	a. Thermostable α-amylase (3000-8000 U/mL) b. Amyloglucosidase (200-U/mL in 50% glycerol) c. Glucose oxidase d. Horse Radish peroxidase e. Potassium dihydrogen phosphate (KH2PO4) f. Sodium hydroxide pellets (NaOH) g. Concentrated HCl h. 4-hydroxybenzoic acid i. Sodium acetate j. 3-(N-morpholino) propane sulfonic acid (MOPS) k. Calcium chloride dihydrate ($CaCl_2$ $2H_2O$) l. Glacial acetic acid m. Dimethyl sulfoxide (DMSO) n. D-Glucose standard o. Control regular maize starch.
Preparation of Reagents	a. MOPS buffer (50 mM, pH 7.0) plus calcium chloride (5 mM) and sodium azide (0.02% w/v): Dissolve 11.55 g of MOPS sodium salt in 900 mL of distilled water and adjust to pH 7.0. Add 0.74 g of calcium chloride dihydrate and 0.2 g of sodium azide and dissolve. Adjust the volume to 1 L. Stable for 6 months at 4 °C.

	b. Sodium acetate buffer (200 mM, pH 4.5) plus sodium azide (0.02% w/v): Add 11.6 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water and adjust to pH to 4.5. Add 0.2 g of sodium azide and dissolve. Bring volume to 1 L. Stable for 6 months at 4 °C. *Warning: Sodium azide should not be added until the pH is adjusted. Acidification of sodium azide releases a poisonous gas*. c. Thermostable α-amylase solution (3000 U/mL): Dilute 1 mLα-amylase solution (in 50% glycerol) to 30 mL with MOPS buffer, (a). Thermostable α-amylase solution is stable up to 3 years when frozen. d. (Note: One unit [U] of α-amylase activity is amount of enzyme required to release 1 mmole p-nitrophenol from "end-blocked" p-nitrophenyl maltoheptaoside in presence of saturating levels of a-glucosidase and amyloglucosidase [i.e., alpha α-amylase assay reagent] at 40°C and pH 6.0.) Thermostable α-amylase solution should be free of detectable levels of free glucose. e. Amyloglucosidase solution (200 U/mL) Use directly without dilution. Solution is viscous; for dis pens ng, use positive displacement dispenser. Amyloglucosidase solution is stable up to 3 years at 4°C. (Note: One unit [U] of enzyme activity is amount of enzyme required to release 1 mmole p-nitrophenol from p-nitrophenyl b-maltoside in the presence of saturating levels of b-glucosidase [i.e., amyloglucosidase assay reagent] at 40°C and pH 4.5.) Amyloglucosidase should be free of detect able levels of free glucose. f. Glucose oxidase–peroxidase–aminoantipyrine reagent (GODPOD)—Mixture of glucose oxidase, 12000 U/L; peroxidase, 650 U/L; and 4-aminoantipyrine, 0.4 mM: Prepare buffer concentrate by dissolving 13.6 g KH2PO4, 4.2 g NaOH, and 3.0 g 4-hydroxybenzoic acid in 90 mL dis tilled H_2O. Adjust to pH 7.4 with either 2M HCl (16.7 mL HCl/100 mL) or 2M NaOH (8.0 g NaOH/100 mL). Dilute solution to 100 mL, add 0.4 g sodium azide, and mix until dissolved. Buffer concentrate is stable up to 3 years at 4°C. To prepare GODPOD, dilute 50 mL buffer concentrate to 1.0 L. Use part of diluted buffer to dissolve the entire contents of vial containing freeze-dried glucose oxidase–peroxidase mixture. Transfer contents of vial to 1 L volumetric flask containing diluted buffer. Reagent is stable 2–3 months at 4°C and 2–3 years at –20°C. Color formed with glucose is stable several hours. (Note: Glucose oxidase must not be contaminated with β- and/or α-glucosidase and chromogen color complex must be stable at least 60 min.).

	g. Aqueous ethanol: About 80% (v/v). Dilute 80 mL 95% ethanol (laboratory grade) to 95 mL with H_2O. h. D-Glucose standard solution (1.0 mg/mL) in 0.02% benzoic acid. Stable for 2 years at room temperature. Before preparing solution, dry powdered crystalline glucose (purity >97%) 16 h at 60°C under vacuum. Dis solve 0.1 g dried glucose, weighed to near est mg, in 100 mL dis tilled water. i. Corn starch: Containing known content of starch (e.g., ca 98% dry weight).
Sample Preparation	1. Mill approx. 50 g of sample (cereal, sago or food product) 2. to pass a 0.5 mm sieve using a centrifugal mill. 3. Homogenize sample by shaking and inversion. 4. Determined moisture content by recording weight loss on storage of flour samples (0.5 g) at 103 ºC for 16 h or until weight stabilization. Use information on final calculation. 5. The amount of D-glucose present in the cuvette should range between 5 and 100 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a D-glucose concentration between 0.05 and 1.0 g/L.
Method of analysis	Run D-glucose working standard solutions (in quadrupli cate), reagent blank (in duplicate), and corn starch with each set of tests. Use reagent blank to zero spectrophotometer (1) Accurately weigh 90–100 mg ground test portion directly into glass test tube. Tap tube gently on laboratory bench to ensure that all particles drop to bottom of tube. (2) When analyzing cereal products containing high levels of glucose [processed cereal products {e.g., breakfast cereals} and all products of unknown or uncertain com position {i.e., products containing free glucose or maltodextrins}], pre-extract 90–100 mg of weighed, ground test sample with 10 mL 80% aqueous ethanol, at ca 80°C for 10 min/extraction. Centrifuge slurry at 1000 ×g and dis card supernatant. Use sediment for analysis.) (3) Add 0.2 mL 80% aqueous ethanol to tube and stir on Vortex mixer to ensure that test portion is wet. (4) Add 3.0 mL thermostable α-amylase, and mix using Vortex mixer to ensure complete dispersion. (5) Immediately place tube in boiling water bath for 2 min, re move from water bath, and mix vigorously on Vortex mixer. Return tube to boiling water bath for additional 3 min and then mix con tents vigorously on Vortex mixer. (Note: Some solids will adhere to side of test tube; however, this will not affect analysis since tube con tents are treated with enzyme in this step.) (6) Place tubes in water bath set at 50°C and let equilibrate 5 min. Add

	4.0 mL 200mM sodium acetate buffer, and 0.1 mL amyloglucosidase solution and vigorously mix con tents on Vortex mixer. Cap tube with a marble and incubate for30 min at 50°C. (7) Quantitatively transfer the entire con tents of test tube to 100 mL volumetric flask. Use water wash bottle to rinse tube contents thoroughly. Dilute to 100 mL with H_2O. (Note: If sample contains a low starch (<10%), adjust volume to 10.0 mL (instead of 100 mL). Take this in in account when performing calculations. (8) Centrifuge an aliquot of this solution at 3,000 rpm for 10 min. or filter aliquot through filter paper. (9) Carefully and accurately dispense supernatant aliquots (0.1 mL) into the bottom of two test tubes. (10) Add 3.0 mL of GODPOD Reagent to each tube (reaction solutions, from test samples and control starch, reagent blanks and D-Glucose standards) and incubate at 50 °C for a further 20 min. (11) Remove the tubes from the water bath and measure the absorbance(A) at 510 nm against reagent blank within 1 h. Use average A values for each test and use in calculations. (12) If the GODPOD absorbance is above 1.1 AU, dilute the sample and repeat the analysis
Calculation with units of expression	The concentration of starch (as is basis) can be calculated as follows $$Total\ starch\ (\%) = \Delta A \times F \times 1000 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$ $$= \Delta A \times \frac{F}{W} \times 90$$ Where: ΔA = sample absorbance at 510 nm read against the reagent blank F =factor to convert absorbance to µg of glucose $$= \frac{100\ \mu g}{Absorbance\ of\ 100\ \mu g\ D-Glucose}$$ 1000 = volume correction (i.e. 0.1 mL taken from 100mL) 1/1000= conversion from □g to mg 100/W= factor to express starch as a percentage of sample weight W = mass of the sample analyzed in mg (“as is”) 162/180= factor to convert free D-glucose, as determined, to anhydrous-D-glucose, as occurs in starch. Starch, % m/m (dry weight basis) = Starch, % w/w (“as is”) × $\frac{100}{100-moisture\ content(\%\ m/m)}$
Sensitivity and Limit of Detection	The sensitivity of the assay is 0.010 AU. This corresponds to a D-glucose concentration of 1.0 mg (or 0.9 mg starch)/L in the sample solution for a maximum sample volume of 1.00 mL. The detection

	limit of 2.0 mg (or 1.8 mg starch)/L is derived from the absorbance difference of 0.020 and a maximum sample volume of 1.00 mL.
Reference	American Association of Cereal Chemists: "Approved Methods of the AACC". Method 76-11, approved October 1976. AOAC Official Method 996.11 Starch (Total) in Cereal Products Amyloglucosidase–α-Amylase Method Final Action 2005 McCleary, B. V., Gibson, T. S. & Mugford, D. C. (1997). Measurement of total starch in cereal products by amyloglucosidase - α-amylase method: Collaborative study. J. AOAC Int., 80, 571-579.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Colour of Gelatinized Alkaline Paste of Sago		
Method No.	FSSAI 03.048:2022	**Revision No. & Date**	0.0
Scope	This method is applicable to determination of the colour of gelatinized paste of Sago (Saboodana) and Palm Sago starch obtained from Sago Palm (*Metroxylon sagu* and *M. rumphii*)		
Caution	Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care		
Principle	The Sago is gelatinized and the color reflectance measured in a Lovibond tintometer		
Apparatus/Instruments	a. Lovibond Tintometer b. Porcelain Cuvette - supplied with the Lovibond Tintometer. c. Mortar and pestle d. Sieve: 250-micron IS Sieve e. Boiling water bath		
Materials and Reagents	Sodium hydroxide AR grade		
Preparation of Reagents	Sodium Hydroxide Solution - approximately 0.5 N prepared by dissolving 20 g of NaOH in distilled water		
Sample Preparation	Take about 100 g of the material and finely powder in a clean pestle and mortar so that the whole of it passes through 250-micron IS Sieve. Place this prepared material in a clean and dry stoppered glass bottle.		
Method of analysis	1. Place about 10 g of the prepared material in a clean and dry neutral glass beaker and add to it 95 mL of water. 2. Heat the beaker with its contents on a boiling water-bath for about 15 minutes with continuous stirring till the material is gelatinized. 3. Add 5 mL of sodium hydroxide solution to the gelatinized paste and stir well. 4. Allow the slurry to cool. 5. Clean the porcelain cuvette with carbon tetrachloride to remove any oily or greasy film on it and allow it to dry. 6. Fill the cuvette with the gelatinized paste and place it in position in the tintometer kept in the vertical position, suitable for measuring reflected light.		

	7. Place alongside of it such red and/or yellow Lovibond glasses as are necessary to match the colour shade of the gelatinized paste, observing the colour of the gelatinized paste and of the combination of Lovibond glasses through the eye piece.
Calculation with units of expression	Report the colour of the gelatinized paste in terms of Lovibond units by summing up individually the values for the red and yellow Lovibond glasses as follows: Colour reading of the gelatinized paste in the porcelain cuvetteon the Lovibond Scale = aR+bY Where: a = the sum total of the various red (R) Lovibond glasses used, b=the sum total of various yellow (Y) Lovibond glasses used
Reference	IS:899-1971 specification for Tapioca Sago (Saboodana) First Revision
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of pH of Aqueous Extract of Edible Starches		
Method No.	FSSAI 03.049:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to determining the pH value of edible starches and their products like sago and Palm Sago starch obtained from Sago Palm (*Metroxylon sagu* and *M. rumphii*)		
Caution	pH electrodes are sensitive. Handle with care		
Principle	An aqueous extract is prepared and the pH measured using a pH meter calibrated with traceable buffer standards		
Apparatus/Instruments	a. Electrodes and pH meter - Calibrated against known buffer solution. b. Conical Flask. c. Top pan balance		
Materials and Reagents	a. Standard pH solutions - of known pH values of 4.5 to 7 b. Distilled water c. Orbital shaker		
Sample Preparation	Take about 100 g of the material and finely powder in a clean pestle and mortar so that the whole of it passes through 250-micron IS Sieve. Place this prepared material in a clean and dry stoppered glass bottle		
Method of analysis	1. Place 10 g of the test sample in a dry conical flask and add 100 mL of cool, recently boiled distilled water. 2. Agitate the flask until an even suspension, free from lumps, is obtained. 3. Allow suspension to stand at 25 °C for 30 minutes, agitating continuously using an orbital shaker or intermittently in such a manner as to keep the starch particles in suspension. 4. Let it stand for 10 more minutes. 5. Decant the supernatant liquid into a clean beaker Immediately determine pH using a pH meter calibrated against known buffer solutions between pH 4.0 and 7.0.		
Calculation with units of expression	Record the pH of the extract		

Reference	Methods of test for edible starches and starch products Part II Chemical methods IS 4706 Part-II (1978) Reaffirmed 2005
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Sulphur Dioxide Content of Edible Starches and their Products		
Method No.	FSSAI 03.050:2022	**Revision No. & Date**	0.0
Scope	This method applies to edible starches and their products like sago and Palm Sago starch obtained from Sago Palm (*Metroxylon sagu* and *M. rumphii*), corn sugars and other water-soluble starch hydrolyzates that contain sulfur dioxide		
Caution	Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care		
Principle	The sample is diluted and treated with sodium hydroxide to release sulfur dioxide. The solution is then acidified and the sulfurous acid determined by titration with a standard iodine solution using starch as an end point indicator.		
Apparatus/Instruments	a. Analytical balance (Readability 0.001 g) b. Micro-burette: 5 mL capacity with 0.01 mL subdivisions and a tolerance of ± 0.01 mL. c. Micro-burette: 10 mL capacity with 0.02 mL subdivisions and a tolerance of 0.02 mL. d. Magnetic stirrer is recommended.		
Materials and Reagents	a. Sodium hydroxide b. Sulfuric acid (96% H_2SO_4, sp g 1.84) c. Potassium iodide d. Crystalline iodine		
Preparation of Reagents	a. Sodium hydroxide solution, 1.0 N: Dissolve 40 g of reagent grade sodium hydroxide (NaOH) in 500 mL of purified water in a 1000 mL volumetric flask. Mix, cool and dilute to volume. b. Sulfuric acid solution, 1.0 N: Add 27.8 mL of concentrated sulfuric acid (96% H2SO4, sp g 1.84) into a 1000 mL volumetric flask containing 500 mL of purified water. Mix, cool and dilute to volume. c. Iodine Solution, Stock, 0.1 N (Note 2): Dissolve 40 g of potassium iodide (KI) in 200 mL of purified water in a 1000 mL volumetric flask. Let the solution come to room temperature, add 12.7 g of resublimed crystalline iodine (I2), stir until completely dissolved, add 3 drops of concentrated hydrochloric acid (37% HCl, sp. gr. 1.19) and dilute to volume with purified water. Mix thoroughly and store in an actinic		

	glass bottle. Standardize as frequently as necessary, so that approximately 25 mL of the iodine solution is equivalent to 25 mL of 0.1 N standard sodium thiosulfate solution using starch indicator for end point detection. d. Iodine Solution, Working Standard, 0.05 N: Using a 50 mL Class A pipet, transfer 50.0 mL of the 0.1 N stock iodine solution into a 100 mL volumetric flask. Dilute to volume with purified water and mix well. Make fresh at least weekly, and store in an actinic glass bottle. e. Starch Indicator Solution, 1%: Slurry 10 g of soluble starch in 50 mL of cold purified water. Transfer quantitatively to 1 L of boiling purified water and stir until completely dissolved. Cool and add 1 g of salicylic acid preservative. Discard after one month.
Method of analysis	1. Weigh accurately 100 g of sample into a 400 mL Erlenmeyer flask. 2. Add sufficient purified water to bring total weight to 200 g 3. Mix the sample and water until the solution is homogenous by warming. 4. Cool the solution, and add 20 ml of 1 N sodium hydroxide solution. 5. Stir 5 minutes and keep it aside for half an hour 6. Add 25 mL of 1.0 N sulfuric acid solution, 10 mL starch indicator solution, and titrate with 0.05 N standardized iodide solution until a light blue color persists. 7. Perform a blank titration using 200 mL of purified water and all reagents.
Calculation with units of expression	Sulfur dioxide (mg/kg), as is $= \frac{(Vs - Vb) \times N \times 0.032 \times 1\,000{,}000}{\text{Mass of sample (g)}}$ Where Vs= Titre value of sample V_b + Titre value of blank N= Normality of Iodine solution 0.032 is the Milliequivalent Weight of Sulfur Dioxide=$\frac{64.07l}{2\times1000}$
Reference	Methods of test for edible starches and starch products Part II Chemical methods IS 4706 Part-II (1978) Reaffirmed 2005
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determining the Density of Malt Extract		
Method No.	FSSAI 03.051:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to malt extract		
Principle	The mass per unit volume of the extract is used to measure the density		
Apparatus/Instruments	a. Analytical balance (Readability 0.0001g) b. Class A Volumetric flask 50 mL		
Method of analysis	1. Dissolve about 25 g of the material, accurately weighed, in about 15 ml of water by warming gently in a 50-ml beaker. 2. Cool and transfer to a tared 50-ml graduated flask 3. Dilute to 50 ml with water. 4. Adjust the temperature to 20 °C and weigh.		
Calculation with units of expression	$\text{Density (g/mL) at 20 °C} = \frac{0.9972 \times M}{49.86 + M - m}$ Where: M = mass, of malt extract taken for the test m = mass, in g, of the malt extract solution.		
Reference	IS 2404: 1993 Reaffirmed 2010 MALT EXTRACT - SPECIFICATION		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Refractive Index of Malt extract		
Method No.	FSSAI 03.052:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for the measurement of Refractive index of malt extract and other liquids.		
Caution	It is extremely important to thoroughly clean the refractometer measuring surface after each use to ensure the most accurate readings and to prevent cross-contamination. Clean the measuring surface with a wet, soft, clean cloth or paper towel. For the most accurate readings, keep the measuring surface clean and free of residue at all times. Do not submerge instrument in liquids. DO NOT use a metal device to transfer samples to the measuring surface. The sample to be tested must not contain solid impurities.		
Principle	A hand refractometer is used to measure the refractive index. By shinning a beam of light through a sample of the liquid, the refractometer measures the amount of liquid that is refracted from the light path due to the constituents in the sample. The device takes the refraction angle and correlates them to already the established refractive index.		
Apparatus/Instruments	a. Abbe's Refractometer: The temperature of the refractometer should be controlled to within ±0.1 °C and for this purpose it should be provided with a thermostatically controlled water-path and a motor driven pump to circulate water through the instrument. The instrument should be standardized, following the manufacturer's instructions, with a liquid of known purity and refractive index or with a glass prism of known refractive index. Distilled water, which has a refractive index 1.333 0 at 20.0°C, is a satisfactory liquid for standardization. b. Light Source If the refractometer is equipped with a compensator, a tungsten lamp or a daylight bulb may be used. Otherwise, a monochromatic light, such as an electric sodium vapour lamp, should be used. c. Micropipette		
Sample Preparation	Filter the sample through a filter paper to remove any solid impurities, if any.		
Method of analysis	1. Adjust the temperature of the refractometer to 20 ±0.1		

	°C. Ensure that the prisms are clean and completely dry 2. Using a micropipette place a few drops of the sample on the lower prism. 3. Close the prisms, tighten firmly with the screw-head, and allow to stand for one or two minutes. 4. Adjust the instrument and light to obtain the most distinct reading possible 5. Determine the refractive index.
Calculation with units of expression	Report the Refractive Index reading rounded off to the third decimal place
Reference	Annex B, IS 2404: 1993 Reaffirmed 2010 Malt Extract - Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Solids in Malt Extract		
Method No.	FSSAI 03.053:2022	**Revision No. & Date**	0.0
Scope	The method is applicable to malt extract and all viscous liquids containing high levels of sugar.		
Caution	*Vacuum oven*: are forbidden for use in unattended or non-working hours. Always wear insulated gloves when removing or placing samples in the heated oven. Open hot ovens with care after release of vacuum. Stand to one side when opening the door to avoid high temperature Exercise extreme caution when opening and closing desiccators		
Principle	The sample is shaken with water and moisture evaporate		
Apparatus/Instruments	a. Flat-Bottom Dish: The dish shall be of nickel or other suitable material not affected by boiling water 7 to 8 cm in diameter and not more than 2.5 cm deep, provided with a short glass stirring rod having a widening flat end. b. Analytical balance (Readability 0.0001 g) c. Vacuum Oven d. Boiling water bath		
Materials and Reagents	Sand: Sand which passes through a 500-micron IS Sieve and is retained on a 180-micron IS Sieve It can be prepared by digestion with concentrated hydrochloric acid, followed by thorough washing with water till free from chlorides then be dried and ignited to dull red heat		
Method of analysis	1. Heat the dish, containing about 20 g of the prepared sand and a stirring rod, in the oven for about one hour. 2. Allow to cool in an efficient desiccator for 30 to 40 minutes. Weigh accurately about 2 g of the material into the tared dish. 3. Add about 5 ml of distilled water in the dish and thoroughly mix the sand with the sample by stirring with the glass rod, smoothing out lumps and spreading the mixture over the bottom of the dish. 4. Place the dish on a boiling water-bath for 30 minutes, 5. Wipe the bottom of the dish and transfer it, with the glass rod, to the vacuum oven maintained at a temperature between 60 and 70 °C and at a pressure of not more than		

	50 mm of mercury. 6. After 2 hours, remove the dish to a desiccator, allow to cool and weigh. 7. Replace the dish in the oven for a further period of one hour, remove to the desiccator, cool and weigh again. 8. Repeat the process of heating, cooling and weighing after every one hour till consecutive weighings do not differ by more than 0.5 mg.
Calculation with units of expression	$$Total\ solids\ \left(\%\frac{m}{m}\right) = \frac{100 \times (M1 - M2)}{M1 - M}$$ Where: M = mass, in g, of the empty dish with the sand and the glass rod. MI = mass, in g, of the contents of the dish before drying, M2 = mass, in g, of the contents of the dish after drying, and
Reference	Annex C, IS 2404: 1993 Reaffirmed 2010 Malt Extract - Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Reducing Sugar in Malt Extract as Maltose		
Method No.	FSSAI 03.054:2022	**Revision No. & Date**	0.0
Scope	The method is applicable for determining the reducing sugar content of malt extract as maltose		
Caution	Concentrated Hydrochloric acid and Sulphuric acid: Handle with extreme care. Both these acids are corrosive and can cause severe burns. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care. Ethyl ether: Extremely volatile and flammable. Handle with extreme care. Irritating to the eyes and the respiratory tract. Diethyl ether can de-fat the skin. Diethyl ether can form explosive peroxides under the influence of light and air. Keep away from heat and light. Handle only inside a fume hood. Store in a tightly sealed container in a cool room (preferably refrigerator) protected from light, moisture and air.		
Principle	The Lane-Eynontitration method is used to determine the concentration of maltose. A burette is used to add the carbohydrate solution being analyzed to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The reducing sugars in the solution react with the copper sulfate present in the flask. Once all the copper sulfate in solution has reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point is recorded.		
Apparatus/Instruments	a. Analytical balance: (Readability 0.0001 g) b. Burette Class A- 50 mL c. Conical Flask d. Volumetric flask 500 mL and 100 mL		
Materials and Reagents	a. Concentrated hydrochloric acid (sp gr 1.16) b. Sodium carbonate c. Benzoic acid		

	d. Ethyl Ether e. Ethyl Alcohol f. Methylene Blue g. Copper sulphate ($CuSO_4$. $5H_2O$) h. Rochelle salt (Potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)) i. Concentrated sulphuric acid (sp gr 1.84) j. Sodium hydroxide k. Lead acetate [Pb $(CH_3COO)_2$ $3H_2O$ l. Disodium hydrogen phosphate, dodecahydrate (Na_2HPO_4, $12H_2O$] m. Potassium oxalate (K_2 C_20_4, H_20) n. Anhydrous D-Glucose: Dry two hours at 100 °C and cool in desiccator before use.
Preparation of Reagents	a. Ethyl Alcohol - 10 percent (v/v). b. Dilute Hydrochloric Acid: 2.5% prepared by mixing 20 mL of and 200 mL of water. c. Neutral Lead Acetate: Dissolve 100 g of lead acetate in distilled water and dilute to one liter. d. Sodium Phosphate-Potassium Oxalate Solution Dissolve 70 g of Disodium hydrogen phosphate, dodecahydrate and 30 g of potassium oxalate in water and dilute to one liter. e. Sodium Hydroxide Solution Approximately 6 N, prepared by dissolving sodium hydroxide analytical reagent (conforming to IS 376 : 1986). f. Sodium carbonate solution 20% (m/v): Weigh 20 g of sodium carbonate and dissolve in water to a final volume of 100 mL g. Stock Solution of Dextrose: Weigh accurately 10 g of anhydrous dextrose into a one-litre graduated flask and dissolve it in water. Add to this solution 2.5 g of benzoic acid, shake to dissolve benzoic acid and make up the volume to the mark with water. After 48 hours this solution should not be used h. Standard Dextrose Solution: Dilute a known aliquot of the above stock solution with water to such a concentration that more than 15 mL but less than 50 mL of it will be required to reduce all the copper in the Fehling's solution s taken for titration. *Note the concentration of anhydrous dextrose in this solution as mg/100 mL Prepare this solution fresh every day.*

	Note - When 10 mL of Fehling's solution are taken for titration, a standard dextrose solution containing 0.11 to 0.30 percent (m/v) of anhydrous dextrose is convenient for use i. Methylene Blue indicator solution - Dissolve 0.2 g of methylene blue in water and dilute to 100 mL j. Fehling's Solution (Soxhlet Modification): Prepared by mixing immediately before use, equal volume of solution A and solution B which are prepared as follows: *Solution A* -Dissolve 34.639 g of copper sulphate ($CuSO_4$. $5H_2O$) in water, add O-5 mL of concentrated sulphuric and dilute to 500 mL in a graduated flask. Filter the solution through prepared asbestos. *Solution B* - Dissolve 173 g of Rochelle salt and 50 g of sodium hydroxide in water, dilute to 500 mL in a graduated flask and allow the solution to stand for two days. Filter this solution through prepared asbestos. k. *Standardization of Fehling's Solution* - Pour the standard dextrose solution into a 50-ml burette. Find the titre (that is, the volume of the standard dextrose solution required to reduce all the copper in 10 ml of Fehling's solution) corresponding to the concentration of the standard dextrose solution from Table 1. (If, for example, the standard dextrose solution contains 167.0 mg of anhydrous dextrose per 100 ml, the corresponding titre would be 30 ml.) Pipette 10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the standard dextrose solution required to effect reduction of all the copper, so that not more than 1 ml will be required later to complete the titration. Heat the flask containing the mixture over a wire gauze. Gently boil the contents of the flask for two minutes. At the end of two minutes of boiling, add, without interrupting boiling, one ml of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add standard dextrose solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears. [The titration should be completed within one minute, so that the contents of the flask boil altogether for three minutes without interruption. *Note: In adding sugar solution to the reaction mixture, the burette may be held in hand over the flask. The burette may be fitted with a small outlet tube bent twice at right angles, so that the body of the burette may be kept out of the steam while adding the sugar solution. Burettes with glass taps are unsuitable for this*

	work, as the taps become heated by the steam and are liable to jam Note the titre. multiply the titre (obtained by direct titration) by the number of milligrams of anhydrous dextrose in 1 ml of the standard dextrose solution to obtain the dextrose factor. Compare this factor with dextrose factor given in Table below. Determine the correction, if any, to be applied to the dextrose factors derived from Table below Deduce maltose factor by reading _ _ corresponding values against corrected dextrose factor from Table below
Sample Preparation	**Preparation of Solution** a. Weigh accurately about 12.5 g of malt extract and transfer to a 250-ml volumetric flask. b. Add 25 ml of the lead acetate solution. c. Make up to volume, mix and filter. Reject the first few drops of the filtrate. d. To 100 ml of the clean filtrate in a 500-ml volumetric flask, add 10 ml of the sodium phosphate-potassium oxalate mixture. e. Make up to volume with water, shake and filter. f. Reject the first few drops of the filtrate and use the clear filtrate for preparation of invert solution **Preparation of Invert Solution** a. To 50 ml of the filtrate in a l00-ml volumetric flask, add 25 ml of water, and 10 ml of concentrated hydrochloric acid. b. Heat on a water bath to 70 °C and regulate heat in such a way the temperature is maintained at 70 °C. c. Place the flask in a water bath, insert a thermometer and heat with constant agitation until the thermometer in the flask indicates 67 °C. d. From the moment the thermometer in the flask indicates 67 °C, leave the flask in the water bath for exactly 5 minutes, during which time the temperature should gradually rise to about 69.5 °C. e. Plunge the flask at once into water at 20 °C. When the contents have cooled to about 35 °C, remove the thermometer from the flask, rinse it f. Add 10 ml of 6 N sodium hydroxide solution for neutralization of acid, leave the flask in the bath at 20 °C for about 30 minutes and then make up exactly to volume with water. g. Mix the solution well and use for titration.
Method of analysis	***Incremental Method of Titration***

	1. Pour the prepared hydrolysate into a 50-mLburette (the same may be filtered if not clear 2. Pipette 10 ml of Fehling's solution into a 300 ml conical flask and run in from the burette 15 ml of the prepared solution. 3. Without further dilution, heat the contents of the flask over a wire gauze, and boil. (After the liquid has been boiling for about 15 seconds, it will be possible to judge if almost all the copper is reduced by the bright red color imparted to the boiling liquid by the suspended cuprous oxide). 4. When it is judged that nearly all the copper is reduced, add 1 ml of the methylene blue indicator solution. 5. Continue boiling the contents of the flask for one to two minutes from the commencement of boiling, and then add the prepared solution in small quantities (1 ml or less at a time), allowing the liquid to boil for about 10 seconds between successive additions, till the blue colour of the indicator just disappears 6. In case there still appears to be much unreduced copper after the mixture of Fehling's solution with 15 ml of the prepared solution has been boiling for 15 seconds, add the prepared solution from the burette in larger increments (more than 1 ml at a time, according to judgement), and allow the mixture to boil for 15 seconds after each addition. 7. Repeat the addition of the prepared solution at intervals of 15 seconds until it is considered unsafe to add a large, increment of the prepared test solution. 8. At this stage continue the boiling for an additional one to two minutes, add 1 ml of methylene blue indicator solution and complete the titration by adding the prepared solution in small quantities (less than 1 ml at time). ***NOTE 1*** *-It is advisable not to add the indicator until the end point has been nearly reached because the indicator retains its full colour until the end point is almost reached and thus gives no warning to the operator to go slowly.* ***NOTE 2*** *- When the operator has had a fair amount of experience with the method, a sufficiently accurate result may often be obtained by a single estimation by the incremental method of titration. For the utmost degree of accuracy of which the method is capable a second titration should be carried out by the standard method of titration.* *9.* Repeat titration twice and calculate the mean of three parallel titrations

Standard method of titration

1. Pipette 10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the prepared solution required to effect reduction of all the copper so that, if possible, not more than one ml will be
2. required later to complete the titration.
3. Gently boil the contents of the flask for two minutes.
4. At the end of 2 minutes of boiling, add without interrupting the boiling, one ml of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add the prepared solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears (see Note 1).
5. The titration should be completed within one minute, so that the contents of the flask boil altogether for 3 minutes without interruption (see Note 2).]

Note:1 The indicator is so sensitive that it is possible to determine the end point within one drop of the prepared test solution in many cases. The complete decolourization of the methylene blue is usually indicated by the whole reaction liquid in which the cuprous oxide is continuously churned up becoming bright red or orange in colour. In case of doubt, the flame may be removed from the wire gauze or one or two seconds and the flask held against a sheet of white paper. The top edge of the liquid would appear bluish if the indicator is not completely decolourized. It is inadvisable to interrupt the boiling as the indicator undergoes back oxidation rather rapidly when air is allowed free access into the flask, but there is no danger of this as long as a continuous stream of steam is issuing from the mouth of the flask.

Note 2 -It should be observed that with both incremental and standard methods of titration, the flask containing the reaction mixture is left on the wire gauze over the flame throughout the titration.

NOTE 4 - The dilution of the test solution (invert solution) should be such that its titre value lies between 15 and 50 ml around 25 ml.

Dextrose/Maltose Factors for 10 mL of Fehling's solution					
Titre value	Dextrose factor*	Maltose factor	Titre value	Dextrose factor*	Maltose factor
15	49.1	81.3	29	50.0	80.0
16	49.2	81.2	30	50.1	79.9
17	49.3	81.1	32	50.2	79.9

	18	49.3	81.0	33	50.3	79.8
	19	49.4	80.9	34	50.3	79.8
	20	49.5	80.8	35	50.4	79.7
	21	49.5	80.7	36	50.4	79.6
	22	49.6	80.6	37	50.5	79.6
	23	49.7	80.5	38	50.5	79.5
	24	49.8	80.4	39	50.6	79.5
	25	49.8	80.3	40	50.6	79.4
	26	49.9	80.2	41	50.7	79.4
	27	49.9	80.1	42	50.7	79.3
	28	50.0	80.0	43	50.8	79.3
				44	50.8	79.2
	*Milligrams of anhydrous dextrose corresponding to 10 mL of Fehling's solution					
Calculation with units of expression	Refer to Table above for the dextrose factor corresponding to the titre and apply the correction previously determined. Deduce maltose factor by reading corresponding values against corrected dextrose factor from Table above Calculate the maltose content of the prepared solution as follows: $\text{m} = \frac{\text{Maltose factor}}{\text{Titre}}$ $\text{Reducing sugar as maltose (\% dry mass basis)} = \frac{m \times 250}{M1\ (100 - M)}$ Where: m=Milligrams of anhydrous maltose present in 1 ml of the prepared solution V=total volume in ml of the prepared solution M1= mass in g of the material used to prepare 250 ml of the solution M= percentage of moisture					
Reference	Annex D, IS 2404: 1993 Reaffirmed 2010 Malt Extract - Specification					
Approved by	Scientific Panel on Methods of Sampling and Analysis					

	Determination of amylose content of rice Spectrophotometric method		
Method No.	FSSAI 03.055:2022	**Revision No. & Date**	0.0
Scope	This is a reference method for the determination of the amylose content of milled rice, non-parboiled. The method is applicable to rice with an amylose mass fraction higher than 5 %. This document can also be used for husked rice, maize, millet and other cereals if the extension of this scope has been validated by the user.		
Caution	Evaporate all solvents in fume hood Sodium hydroxide is caustic. Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs. Prolonged or repeated skin contact may cause dermatitis. Handle with care		
Principle	Rice is ground to a very fine flour to break up the endosperm structure and the flour is then defatted. A test portion is dispersed in a sodium hydroxide solution. An aliquot portion is taken to which an iodine solution is added. The absorbance, at 720 nm, of the colour complex formed is then determined using a spectrophotometer. The amylose mass fraction of the sample is then read from a calibration graph, which is prepared using mixtures of potato amylose and amylopectin to make allowance for the effect of amylopectin on the colour of the amylose–iodine complex of the test solution.		
Apparatus/Instruments	a. Laboratory blender. b. Grinder, capable of reducing uncooked milled rice to flour that will pass through a 150 µm to 180 µm (100 mesh to 80 mesh) sieve. A cyclone mill with 0,5 mm screen is recommended. c. Sieve, size 150 µm to 180 µm (100 mesh to 80 mesh). d. Spectrophotometer, with matching cells, usually of path length 1 cm, capable of measuring absorbance at 720 nm. e. Extraction apparatus, capable of refluxing samples with methanol at a rate of 5 to 6 droplets per second. f. Volumetric flasks, 100 ml. g. Boiling water bath. h. Conical flasks, 100 ml. i. Analytical balance, capable of weighing to the nearest 0.000 1 g.		
Materials and Reagents	a. Methanol (85 %).		

	b. Ethanol, 95 %. c. Sodium hydroxide d. Sodium dodecylbenzene sulfonate e. Acetic acid f. Potassium iodide g. Potato amylose
Preparation of Reagents	a. Sodium hydroxide (1M). Weigh 40 g of NaOH pellets and dissolve in water by cooling. Make the volume to one litre b. Sodium hydroxide (0.09M): Weigh 3.6g of NaOH pellets and dissolve in water by cooling. Make the volume to one litre. c. Sodium hydroxide, for protein removal, 3 g/l solution. d. Detergent solution. Dissolve sodium dodecylbenzene sulfonate corresponding to a concentration of 20 g/l. Just before use, add sodium sulfite to a final concentration of 2 g/l. e. Acetic acid (1M) f. Iodine solution. Weigh, to the nearest 5 mg, 2.000 g of potassium iodide in a weighing bottle fitted with a stopper. Add sufficient water to form a saturated solution. Add 0.200 g of iodine, weighed to the nearest 1 mg. When all the iodine has dissolved, transfer the solution quantitatively to a 100 ml volumetric flask make up to volume with water and mix. Note: Prepare a fresh solution on each day of use and protect it from light.
Preparation of amylose and amylopectin standard	*Preparation of standard: Stock potato amylose suspension, free of amylopectin: 1 g/l.* *1.* Defat the potato amylose by refluxing with methanol for 4 h to 6 h in an extractor at a rate of 5 to 6 droplets per second. 2. The potato amylose should be pure and should be tested by amperometry or potentiometric titration. Pure amylose should bind 19 % to 20 % of its own mass of iodine. 3. Spread the defatted potato amylose on a tray and leave for two days in a fume hoof to allow evaporation of residual methanol and for moisture content equilibrium to be reached. 4. Weigh 100 mg ± 0,5 mg of the defatted and conditioned potato amylose into a 100 ml conical flask.

	5. Carefully add 1 ml of ethanol, rinsing down any potato amylose adhering to the walls of the flask. 6. Add 9.0 ml of 1 M sodium hydroxide solution and mix. 7. Then heat the mixture on a boiling water bath for 10 min to disperse the potato amylose. 8. Allow to cool to room temperature and transfer into a 100 ml volumetric flask. 9. Make up to volume with water and mix vigorously. 10. One ml of this stock suspension contains 1 mg of potato amylose *Stock amylopectin suspension, 1 g/L* 1. Prepare the stock from milled glutinous (waxy) rice with a starch content known to consist of at least 99 % by mass of amylopectin. 2. Steep the milled glutinous rice and blend in a suitable laboratory blender to a finely divided state. 3. Remove protein by exhaustive extraction with a detergent solution or, alternatively, with a sodium hydroxide solution 4. Wash and then defat by refluxing with methanol (5.1) as described for amylose. 5. Spread the deproteinated and defatted amylopectin on a tray and leave for two days to allow evaporation of residual methanol and for moisture content equilibrium to be reached. 6. Carry out the steps 4-9 as above, but with amylopectin instead of amylose. 7. 1 ml of this stock suspension contains 1 mg of amylopectin. 8. The iodine binding capacity of amylopectin should be less than 0.2 %
Sample Preparation	1. In the cyclone mill grind at least 10 g of milled rice to very fine flour that will pass through the sieve (size 150 µm to 180 µm). 2. Defat the flour by refluxing with methanol for 4 h to 6 h in an extractor at a rate of 5 to 6 droplets per second. Note Lipids compete with iodine in forming a complex with amylose and it has been shown that defatting the rice flour effectively reduces lipid interference. 3. After defatting, spread the flour in a thin layer in a dish or watch glass and leave for two days to allow evaporation of residual methanol and for moisture

	content equilibrium to be reached. Use of a fume hood, when evaporating the methanol.
Method of analysis	1. Weigh 100 mg ± 0,5 mg of the defatted test sample into a 100 ml conical flask. 2. To this test portion, carefully add 1 ml of ethanol, rinsing down any of the test portion adhering to the walls of the flask and shaking slightly to make all the sample wet. 3. Add 9.0 ml of 1M sodium hydroxide solution and mix. 4. Then heat the mixture on a boiling water bath for 10 min to disperse the starch. 5. Allow to cool to room temperature (25±3 °C) and transfer to a 100 ml volumetric flask. 6. Make up to volume with water and mix vigorously. 7. Prepare a blank solution using the same procedure and the same quantities of all the reagents as in the determination, but using 5.0 ml of 0.09 M sodium hydroxide solution instead of the test solution. 8. Pipette 5.0 ml aliquot of the test solution into a 100 ml volumetric flask containing about 50 mL of water 9. Add 1.0 ml of acetic acid and mix. 10. Then add 2.0 ml of iodine solution make up to the mark with water and mix. 11. Allow to stand for 10 min. Measure the absorbance at 720 nm against the blank solution using the spectrophotometer 12. Carry out two determinations on separate test portions taken from the same test sample. Preparation of the calibration graph: 1. Mix volumes of the potato amylose and amylopectin stock suspensions and of the 0.09 M sodium hydroxide solution in accordance with Table shown below 2. Pipette a 5.0 ml aliquot of each calibration solution into a series of 100 ml volumetric flasks each containing about 50 ml of water. 3. Add 1.0 ml of acetic acid and mix. Then add 2,0 ml of iodine solution make up to the mark with water and mix. Allow to stand for 10 min. Measure the absorbance at 720 nm against the blank solution using the spectrophotometer.

	4. Prepare a calibration graph by plotting absorbance against the amylose mass fraction, expressed as a percentage.

Set of calibration solutions			
Amylose mass fraction in milled rice (%, dry matter basis)	Potato amylose (mL)	Potato amylopectin (mL)	Volume of 0.09 NaOH (mL)
0	0	18	2
10	2	16	2
20	4	14	2
25	5	13	2
30	6	12	2
35	7	11	2
These values have been calculated on the basis of an average starch mass fraction of 90 % in milled rice.			

Calculation with units of expression	The amylose mass fraction, expressed as a percentage on the dry basis, shall be obtained by referring the absorbance of the test sample to the calibration graph. Take the arithmetic mean of the two determinations as the result.
Reference	ISO 6647-1 Third edition 2020-07 Rice — Determination of amylose content — Part 1: Spectrophotometric method with a defatting procedure by methanol and with calibration solutions of potato amylose and waxy rice amylopectin
Approved by	Scientific Panel on Methods of Sampling and Analysis

<table>
<tr><td>  </td><td colspan="3">Determination of Alkali Spreading Value of Rice Kernels</td></tr>
<tr><td>Method No.</td><td>FSSAI 03.056:2022</td><td>Revision No. & Date</td><td>0.0</td></tr>
<tr><td>Scope</td><td colspan="3">Alkali spreading value directly determines the cooking quality of rice varieties. The method is applicable to all rice varieties</td></tr>
<tr><td>Principle</td><td colspan="3">When rice is treated with dilute alkali, the starch molecules present in rice get degraded resulting in disintegration of the grain. Depending upon the variety, the changes in the grain shape may vary from no apparent effect to a completely dispersed grain. The changes are recorded using a seven-point scale. The waxy and the low amylose rice grains disintegrate fast whereas the high amylose grains retain the shape</td></tr>
<tr><td>Apparatus/Instruments</td><td colspan="3">a. Petri plates
b. Incubator set at 30 °C</td></tr>
<tr><td>Materials and Reagents</td><td colspan="3">a. 1.7% potassium hydroxide solution</td></tr>
<tr><td>Preparation of Reagents</td><td colspan="3">a. 1.7% potassium hydroxide solution: Dissolve 1.7 g of KOH pellets in 100 mL of distilled water</td></tr>
<tr><td>Method of analysis</td><td colspan="3">1. Randomly select six whole grains of rice
2. Place the grains in a glass petri-dish containing 10 ml of 1.7% potassium hydroxide solution.
3. Cover the petri-dishes and incubate for 23 hours at 30 °C. The degree of spreading due to alkali is measured by using a seven-point numerical scale as presented in Table below
4. The degree of spreading is measured using a seven-point scale as follows:
<table>
<tr><th>Features</th><th>Scale</th></tr>
<tr><td>Grain not affected</td><td>1</td></tr>
<tr><td>Grain swollen</td><td>2</td></tr>
<tr><td>Grain swollen, collar incomplete and narrow</td><td>3</td></tr>
<tr><td>Grain swollen, collar complete and wide</td><td>4</td></tr>
<tr><td>Grain split or segmented, collar complete and wide</td><td>5</td></tr>
<tr><td>Grain dispersed, merging with collar; and</td><td>6</td></tr>
</table></td></tr>
</table>

	Grain completely dispersed and intermingled | 7 Source: IRRI (1996) Standard Evaluation System for Rice. International Rice Research Institute, Los Banos, Philippines
Calculation with units of expression	Alkali Spreading Value is expressed from a scale of 1-7
Reference	Little RR, Hilder GB, Dawson EH, 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chemistry, 35: 111-126.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Measurement of Rice grain Dimensions: Length, Breadth, Length/Breadth Ratio		
Method No.	FSSAI 03.057:2022	**Revision No. & Date**	0.0
Scope	Grain dimensions or grain size and shape (length-width ratio) are a very stable varietal property. The method is applicable to all rice grains		
Principle	The grain dimensions (length and breadth) are directly measured using a pair of slide calipers. The ratios are then calculated mathematically.		
Apparatus/Instruments	Slide calipers		
Method of analysis	1. Randomly select 10 whole kernels of rice in three sets 2. Open the jaws of the slide calipers and place the grain or commodity between the jaws. 3. Read the dimension (length and breadth) in mm from the scale. 4. Obtain the average length and width of the grains in mm.		
Calculation with units of expression	$Average\ length - breadth\ ratio = \frac{Average\ grain\ length\ (mm)}{Average\ grain\ breadth\ (mm)}$		
Reference	Anjum, K.I. and Hossain, M.A. (2019) Nutritional and cooking properties of some rice varieties in Noakhali region of Bangladesh, Res. Agric. Livest. Fish. 6, No. 235-243.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Measurement of Volume Expansion Ratio and Kernel Elongation Ratio		
Method No.	FSSAI 03.058:2022	**Revision No. & Date**	0.0
Scope	Volume expansion ratio and elongation ratio cooking quality parameters that are directly related to the physical and chemical characteristics of the starch in the endosperm. The method described is applicable to all rice varieties		
Principle	The volume expansion ratio of the samples is determined by water displacement method by using a measuring cylinder. Elongation ratio of cooked kernels is determined by dividing the length of cooked kernel to length of uncooked kernel, which are measured using calipers.		
Apparatus/Instruments	a. Analytical balance (Readability 0.01g) b. Water bath c. Slide calipers d. Microscale e. Graduated measuring cylinder (Class A)		
Method of analysis	*Volume expansion ratio* 1. Weigh 5 g rice grains and pour into a measuring cylinder containing 15 ml of water 2. Observe the total volume. 3. The initial increase in volume after adding 5 g of rice was recorded (Y) and soaked for 10 min. 4. The rice grain sample is cooked for 20 min in a water bath at 90 °C. 5. All the 5 g of cooked rice are placed in 50 mL water taken in 100 mL measuring cylinder 6. The increase in volume of water is measured (X). 7. The volume raise was recorded (X-50). 8. The volume expansion ratio is calculated *Kernel elongation ratio* 1. Measure the kernel length of 10 whole rice kernels 2. Measure the length of the 10 whole rice kernels after cooking (20 min in a water bath at 90 °C ratio) using a micro-scale or slide calipers 3. Kernel elongation ratio is calculated by dividing the average length of cooked kernel by the average length of the raw (uncooked) rice		

Calculation with units of expression	Volume Expansion Ratio $= \frac{X - 50}{Y - 15}$ where: (X–50) is the volume of cooked rice (ml) (Y-15) is the volume of raw rice (ml) Kernel Elongation Ratio $= \frac{Average\ length\ of\ the\ cooked\ kernel\ (mm)}{Average\ lenght\ of\ the\ uncooked\ kernel (mm)}$
Reference	Juliano, B.O., and Perez (1984) Results of a collaborative test on the measurement of grain elongation of milled rice during cooking. J. Cer. Sci 2. 281-292
Approved by	Scientific Panel on Methods of Sampling and Analysis