MANUAL OF METHODS

OF

ANALYSIS OF FOODS

CEREAL AND CEREAL PRODUCTS

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA
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MANUAL FOR ANALYSIS OF CEREAL AND CEREAL PRODUCTS

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Note: The test methods given in the manuals are validated/ standardized test methods. However, it would be the responsibility of the respective testing laboratory to confirm that the above methods are validated in its laboratory and gives proper result in their laboratory.

MANUAL FOR ANALYSIS OF CEREAL AND CEREAL PRODUCTS

Standards for cereals, pulses and their products are laid down in Section 2.4 of Food Safety and Standards (Food Product Standards and Food Additives) Regulations, 2011. These include standards for food grains, their milled products and processed cereal products. In addition standards for malted foods and solvent extracted edible oilseed flours are also included under this item.

1.0 FOOD GRAINS

1.1 DEFINITION OF REFRACTIONS

Refractions mean all components of food grains, which differ from normal grains such as foreign matter, other food grains, damaged grains, weevilled grains, broken, shriveled grains etc. The definition of various refractions is given under 'Explanation" in 2.4.6.15 for items 2.4.6 (2-14) in Food Safety and Standards (Food Product Standards and Food Additives) Regulations, 2011. Additional definitions are:

(1) 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*.

(2) 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 Ergotoxin and occurs in rye, millets and wheat (Ref: - IS: 8184 – 1976 Method for determination of Ergot in Food grains).

(3) Filth – Any objectionable matter contributed by animal contamination of the product such as rodent, insect or bird matter, or any other objectionable matter contributed by insanitary conditions

(a) Heavy Filth – Heavier filth material separated from product by sedimentation based on different densities of filth, food particles and immersion liquids such as Chloroform etc. Examples of such filth are insect and rodent excreta pellets and pellet fragments, sand and soil.

(b) Light filth – Lighter filth particles that are oleophilic and are separated from product by floating them in an oil – aqueous liquid mixture. Examples are insect fragments, whole insects, rodent hairs and feather barbules.

(c) Sieved filth – Filth particles of specific size ranges separated quantitatively

from product by use of selected sieve mesh sizes.

(Ref: - AOAC 17th edn, 2000, Official method 970. 66 Light and Heavy Filth)

1.1.1 Equipment

(a) Balance – sensitivity 1 mg

(b) I. S sieves of round holes having following aperture size:

Top	4.0 mm
Second from top	3.35 mm
Third from top	1.70 mm
Fourth from top	1.0 mm

A solid bottom pan at the bottom

(c) Enameled Trays – Flat type 30 cm diameter with raised rims

(d) Small scoop

(e) Forceps

(f) Magnifying glass with a handle of about 7.5 cm and a magnification of 10×.

1.1.2 Procedure

Examine the test sample for its general condition, such as appearance: freedom from moulds, insect infestation, off odour, poisonous and deleterious matter.

1.2 DETERMINATION OF FOREIGN MATTER

Determine foreign matter by transferring 500 gm of the sample over the set of sieves arranged in such a way that the sieve with the largest perforation comes at the top and those with smaller perforations are placed in the descending order of their sizes and the solid pan at the bottom. Agitate the sample thoroughly to strain out the foreign matter at various levels. 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. 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. Separate the sieves after straining and pick up all foreign matter and add it to the foreign matter collected on the bottom pan. Weigh the total foreign matter of the bottom pan and calculate the percentage.

In the case of rice, millets and smaller sized grains the quantity of sample for test should be 250 gm.

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.

1.3 DETERMINATION OF MINERAL MATTER

Separate the foreign matter into mineral (inorganic) and organic foreign matter by transferring the entire foreign matter collected into a beaker containing carbon tetrachloride. The inorganic extraneous matter (mineral matter) will settle down, which can be separated from organic foreign matter. Remove the organic foreign matter, dry at 100°C and weigh. Calculate the percentage. The remaining amount shall be the mineral matter.

1.4 DETERMINATION OF REFRACTIONS OTHER THAN FOREIGN MATTER

Mix the contents of the four sieves freed from foreign matter together and spread out evenly on a flat smooth surface. From this spread take exactly the specified quantity required for analysis as indicated below from different parts by quartering the sample. 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 under 15.2.4.6 (2-14) of Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. Weigh each refraction and calculate the percentage.

1.4.1 Quantity of sample to be taken for determining refractions other than foreign matter

Bolder grains such as: Wheat/Maize/Barley/Whole pulses:	50 gm
Smaller grains such as: Rice/Split pulses/millets:	20gm

(Ref: IS 4333 (Part 1): 1996 Methods of analysis for Food grains Part I Refractions)

1.5 DETERMINATION OF RODENT EXCRETA AND HAIR

1.5.1 Rodent Excreta

Weigh 50 gm of sample in a 250 mL hooked-lipped beaker. Add chloroform within 1 cm of the top, mix thoroughly and let settle for 30 minutes stirring surface layer occasionally. Carefully decant Chloroform and floating tissue on to a Buchner funnel without disturbing the heavy residue at the bottom of beaker. Before decanting, take care that the floating layer has not become so compact as to render this operation difficult. Add Carbon tetrachloride equal to the amount of Chloroform and sample left in the beaker, let settle again and decant as before. Repeat the process with mixture of equal parts of Chloroform and Carbon tetrachloride until very little tissue remains in the beaker. Do not decant any rodent excreta fragments that may be present. Wash residue in beaker on to a 7 cm ruled paper with stream of Chloroform or Carbon tetrachloride and examine microscopically. Retain decanted floating tissues for analysis of light filth.

1.6 DETERMINATION OF LIGHT FILTH

Draw air through the Buchner funnel until solvent evaporates. Air dry overnight or dry in oven at 80°C. (In oven drying phosgene is liberated and adequate ventilation must be provided). Transfer residue to 1 L Wildman trap consisting of 1 or 2 L Erlenmeyer flask into which is inserted close fitting rubber stopper supported on a stiff metal rod 5 mm in diameter and about 10 cm longer than the height of the flask. The rod is threaded at the lower end and furnished with nuts and washers to hold it in place on stopper. The lower nut and washer is sunk in the rubber to prevent striking flask.

Add 100 mL 60% Isopropanol saturated with Heptane (To 600 mL isopropanol add 45 mL heptane and 430 mL water, mix and let stand overnight. Siphon from below surface) and mix thoroughly. Wash down sides of the flask with isopropanol–heptane solution until about 400 mL is added and soak for 30 minutes. Trap off twice with 20-30 mL of heptane for each trapping and 60% isopropanol saturated with heptane as the liquid extraction medium. Filter and examine each trapping microscopically

(Ref: - AOAC 17th edn, 2000, Official method 941.16 Filth in grain products and brewers grits)

1.6.1 Light Filth in Whole Wheat Flour

1.6.1.1 Principle

Whole wheat flour is digested without effect on insect exoskeleton or mammalian hair contaminants. These oleophilic filth elements are separated from non oleophilic food products by attraction to the oil phase of an oil –aqueous mixture. The oil phase is trapped off, filtered and examined microscopically for filth elements.

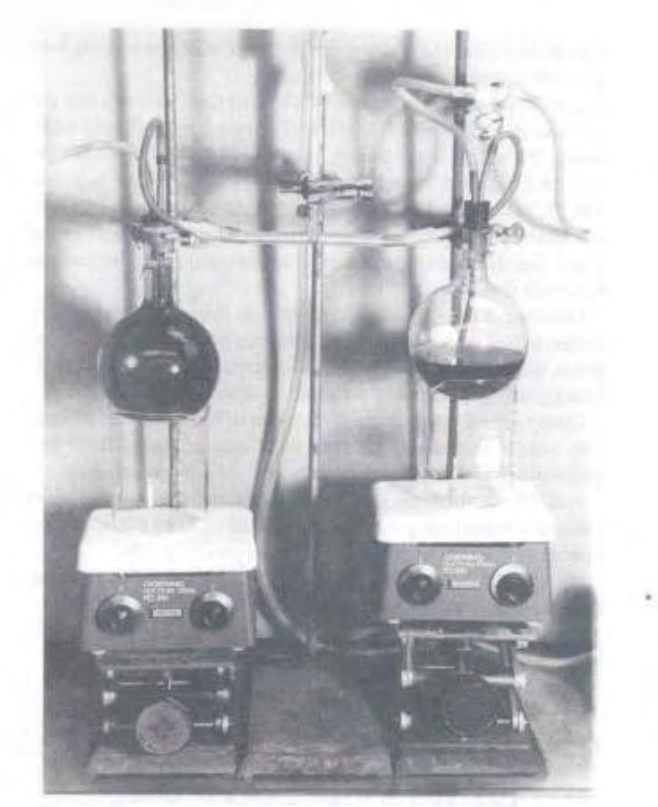
1.6.1.2 Apparatus

(a) Sieve - (1) 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

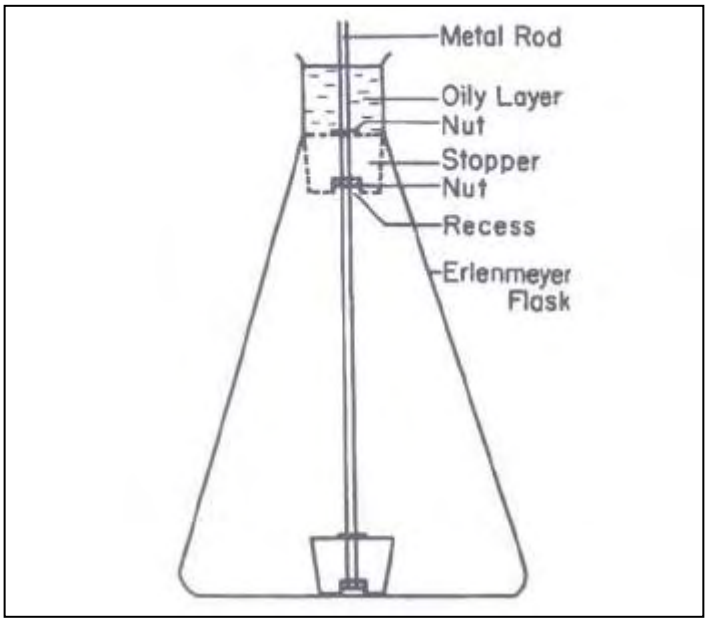
(b) Reflux apparatus – see figure below

(c) Wildman trap flask – see figure below:

(d) Filter paper – Ruled - Use smooth, high wet strength filter paper ruled with oil,- alcohol, and water- proof lines 5 mm apart. S & S No 8 is satisfactory.



Reflux Apparatus (Solvent saver apparatus)



Wildman Trap Flask

1.6.1.3 Reagents

(a) 3 %HCl solution –Add 24 mL concentrated HCl to 776 mL water.

(b) Isopropanol solution: (1) 100% (2) 40% aqueous solution

(c) Mineral oil – Paraffin oil, white, light , sp gr. 0.840 – 0. 860. Request supplier to provide certificate of analysis.

(d) Tween 80 – 40% isopropanol solution – To 40 mL of Polysorbate 80 add 210 mL isopropanol, mix and filter.

(e) EDTA–40 % isopropanol solution – Dissolve 5 gm Tetrasodium EDTA in 150 mL water, add 100 mL isopropanol, mix and filter**.**

1.6.1.4 Isolation

Add 800 mL of 3% HCl solution to a 2 L beaker. Place on preheated hot plate and magnetically stir so stirring bar is visible in vortex (Teflon covered bars approx 47 mm long × 9 mm outer diameter for use with hot plate having continuous variable heat and speed control)

Accurately weigh 50 gm of wheat flour to nearest 0.5 gm into a 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. Remove watch glass and boil gently for 15 minutes while magnetically stirring. Use clean sieve of 8 inch diameter, appropriate mesh size as prescribed (plain sieve). Hold sieve under aerator, which produces an even fine spray of water at 30° angle. Use of sieve handle or similar device helps maintain proper angle of sieve. Pour well mixed sample portion wise (not so much that clogging or excessive foaming results) on to sieve so that moderate pressure spray of water contacts material on sieve. Increase water pressure to achieve maximum spray action on sieve, but not so violent that sample froths over lip of sieve. Keep sample material washed to lower inside edge of sieve until majority of detergent foaming subsides and through water is essentially clear. Repeat portion wise addition of sample and wash sample container thoroughly on final addition. Continue washing on sieve until all detergent foaming subsides and through water is clear. Wash residue to side of sieve with hot tap water and rinse residue with 100% Isopropanol.

Quantitatively transfer residue to original beaker, washing with 100% Isopropanol. Add 100% Isopropanol to 400 mL mark on beaker and boil gently for 5 minutes using reflux apparatus inserted into beaker top. Remove beaker from reflux apparatus and quantitatively, transfer beaker contents to sieve.

Wet sieve with gentle stream of hot water until rinse is clear. Wash residue on sieve with 40% Isopropanol and quantitatively transfer residue to Wildman trap flask using 40 % Isopropanol. Dilute to 600 mL with 40% Isopropanol and boil gently for 5 minutes with magnetic stirring. Remove from heat. Add 65 mL mineral oil and magnetically stir 3 minutes. Let stand for 1-2 minutes after stirring.

Add mixture of Tween 80-40% Isopropanol solution and 5 mL EDTA-40% Isopropanol solution slowly down stirring rod. Hand stir gently for 30 seconds. Let stand undisturbed for 1-2 minutes. Fill flask with 40% Isopropanol, clamp rod and let stand for 30 minutes. Stir bottom contents every 5 minutes for first 20 minutes and leave undisturbed for final 10 minutes. Spin stopper (wafer) to remove any trapped residue and trap off into 400 mL beaker using 40% Isopropanol to rinse neck of flask. Add 40 mL mineral oil to flask and hand stir 15 seconds with gentle up and down motion. Fill flask with 40% Isopropanol and let stand for 20 minutes. Spin stopper and trap off as before, rinsing neck with 100% Isopropanol. Filter beaker contents through

filter and examine microscopically at 30 ×.

(Ref: - AOAC 17th edn, 2000, Official method 993.26 Light filth in Whole Wheat Flour)

2.0 DETERMINATION OF MOISTURE

2.1 Apparatus

(a) Grinding Mill - capable of grinding rapidly and uniformly without development of appreciable heat. The ground material should pass through 1.0 mm I.S sieve. Cold grinding mills can be used instead.

(b) Moisture dishes – made of Aluminium or stainless steel approx 7.5 mm wide and 2.5 mm deep with tight fitting lids.

(c) Electric oven – well ventilated and thermostatically controlled to maintain temperature between 130 – 133°C.

(d) Desiccators containing an effective desiccant.

2.2 Procedure

Mix the test sample and grind suitable quantity to give sufficient ground material for replicate determination. Ensure that the sample is neither too coarse nor too fine and passes through the 1.0 mm sieve.

Weigh accurately about 5 gm of sample in a previously dried and tared dish and place the dish with its lid underneath in the oven for 2 hours. The time should be reckoned from the moment the oven attains 130°C after the dishes have been placed. Remove the dish after 2 hours, cool in the desiccator and weigh. The dish should be placed back in the oven at half hour intervals till constant weight is achieved. The specification for the size of the dish should also be included.

2.3 Calculation

$$\text{Moisture \%} = \frac{(W1 - W2) \times 100}{W1 - W}$$

Where,

W1 = Weight in gms of the dish with the material before drying

W2 = Weight in gms of the dish with the material after drying

W = Weight in gms of the empty dish

(Ref: - IS 4333 (Part II) : 2002 Methods of Analysis of food grains Part II Moisture)

3.0 DETERMINATION OF URIC ACID

(AOAC Method No. 970.24 can be used with applicable levels of more than or equal to 4 mg/100g.)

3.1 Principle

The method is based on the precipitation of proteins and treatment of protein free filtrate with Benedict's uric acid reagent and sodium cyanide and measuring the resultant blue colour colorimetrically.

3.2 Apparatus

(a) Photo electric colorimeter/spectrophotometer
(b) Volumetric flask – 50 mL capacity

3.3 Reagents

(a) Sodium tungstate solution - 10 % (w/v)
(b) Standard Sulphuric Acid solution – 0.667 N
(c) Benedicts Uric acid reagent – Dissolve 100 gm of pure Sodium tungstate in 600 mL water. Add 5 gm 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.
(d) Sodium cyanide solution – 5% containing 2 mL of ammonia per L. This solution requires to be prepared fresh after about six weeks.
(e) Standard Uric acid solution stock solution – Dissolve 9 gm 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.
(f) Working Standard uric acid solution – 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.

3.4 Procedure

Weigh 50 gm sample and grind it finely. Take between 4-20 gm powder expected to contain 1 to 5 mg uric acid and suspend in 200 mL water. Allow the mixture to stand for 2 hours and then mix in a Waring blender for 10 minutes and centrifuge at about 2000 r.p.m. for 10 minutes. 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. 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. Determine the intensity of the colour in a colorimeter using 520 nm filter. The optical density obtained can be recorded as OD1."

Take 10 mL of standard uric acid solution containing 0.2 mg of uric acid in a 50mL flask, add 5 mL of sodium cyanide followed by 1 mL of Benedicts uric acid reagent. Dilute to mark after 5 minutes and determine the intensity of colour in a photoelectric colorimeter using a 520 nm filter or spectrophotometer (OD2).

A parallel test using the same quantity of good uninfested sample as the sample under test should be run as a negative control.

$$\text{Calculation} = \frac{(OD1 - OD2) \times 10 \times 2}{\text{Weight of sample in gm}}$$

(Ref: IS 4333 (Part 5) 1970 – Methods of Analysis for Food grains Part 5)

4.0 TEST FOR PRESENCE OF ERGOT IN FOOD GRAINS

4.1 Reagents

(a) Petroleum ether – 40– 60°C

(b) Solvent ether

(c) Dilute Ammonia 10% (v/v)

(d) Tartaric acid solution – 1% (freshly prepared).

(e) *p*-dimethyl amino benzaldehyde (PDAB) – Dissolve 0.125 gm 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% Ferric chloride solution and let it stand for 24 hours before use.

4.2 Apparatus

(a) Grinding mill

(b) Electric shaker

4.3 Procedure

Grind about 50 gm of sample in the grinding mill to a fine powder. Take 10 gm of powdered sample in a stoppered conical flask. Add sufficient petroleum ether and shake for half an hour in the electric shaker. Allow to settle and decant off the petroleum ether. Dry the material in air. Add to the material 8 mL of dilute ammonia and sufficient quantity of solvent ether. Again shake for 30 minutes. Filter ether portion into a beaker and concentrate to a small volume. 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 p-dimethyl benzaldehyde solution. The appearance of blue colour indicates presence of Ergot.

(Ref: - IS 8184 :1976 Method of determination of Ergot in Food grains)

5.0 DETERMINATION OF HYDROCYANIC ACID IN BEANS

5.1 Principle

The glucosides are hydrolysed and the liberated Hydrocyanic acid steam distilled and titrated with Silver nitrate in an ammonical medium in the presence of potassium iodide, the hydrocyanic acid forming the soluble complex $Ag(CN)_2$. The end point of the titration is characterized by the appearance of permanent turbidity due to precipitation of silver iodide.

5.2 Apparatus

(a) Mechanical grinding mill

(b) Sieve with 1 mm aperture

(c) Balance

(d) Volumetric flask 250 mL

(e) Pipette 100 mL

(f) Steam Distillation apparatus

5.3 Reagents

(a) Ammonium hydroxide solution – Approx – 6M prepared by diluting concentrated ammonia solution (0.9 gm/mL) with an equal volume of water.

(b) Potassium Iodide solution - 5%

(c) Standard Silver Nitrate solution – 0.02 M

(d) Sodium hydroxide solution: 0.5 gm in 20 mL water

5.4 Procedure

Grind a small quantity of the sample and reject it. Then grind adequate quantity of the remaining sample to pass through a 1.0 mm sieve. Weigh 20 gm of ground sample, transfer to 1 L distillation flask or 800 mL Kjeldahl flask, add 200 mL water and let stand for 2 hours. Autolysis should be conducted with apparatus completely connected for distillation. Steam distill, collect 150- 160 mL distillate in Sodium hydroxide solution (0.5 gm in 20 mL water) and dilute to definite volume i.e 250 mL. Take 100 mL, add 8 mL 6M ammonium hydroxide and 2 mL of Potassium iodide solution and titrate with 0.02 M Silver nitrate until permanent turbidity appears. For easy recognition of the end point of titration it is recommended that a black background be used.

5.5 Calculation

1 mL 0.02 M Silver Nitrate = 1.08 mg of HCN

(Ref: AOAC 17th edn Official method 915. 03 Hydrocyanic acid in Beans/IS 11535:1986/ISO 2164- 1975 Method of test for determination of glycosidic hydrocyanic acid in pulses)

6.0 DETERMINATION OF AFLATOXIN

Refer to 5.0 Determination of Aflatoxins in Corn and Peanut Powder / Butter - Liquid Chromatographic method in **Manual For Methods of Analysis of Mycotoxins**

7.0 DETERMINATION OF DEOXYNIVALENOL (DON) – Refer to Manual for analysis of Mycotoxins

Performance characteristics to be defined in the current method as proposed below:

Repeatability – Recommended limit 0.1%

Reproducibility - Recommended limit 0.3%

Limit of detection -25 mcg/kg.

Limit of quantification: 50 mcg/kg.

Repeatability: At DON concentrations ranging between 50 and 250 µ/kg , the relative absolute difference between two single test results should not be greater than 33% of their mean value.

Intermediate Reproducibility:

At a DON concentration level close to 100 mcg/kg , the relative absolute difference between two single test results should not be greater than 45% of their mean value.

Suggestion: To use ELISA based method for quantitative determination of DON.

7.1 Principle

Deoxynivalenol (DON) is extracted from cereals with distilled water. The sample extracts are filtered and added (along with 5 DON controls) to mixing wells containing enzyme-labeled DON (conjugate). Then the solutions are transferred to antibody-coated wells, where free DON in the samples and controls is allowed to compete with enzyme-labeled DON (conjugate) for the antibody binding sites. After incubation, the wells are washed to remove all unbound materials, and a substrate is added which reacts with the bound conjugate to produce a blue color. More blue color in the well means less DON present in the sample extract. Quantitation is performed by measuring the absorbances in a microwell reader fitted with a 650 nm filter and comparing the readings against a standard curve established with the 5 DON controls.

8.0 ANALYSIS OF ATTA (WHEAT)

Preparation of sample

Invert and roll container several times to ensure homogeneous mixture. Avoid extreme temperatures and humidities while opening containers for analysis.

Keep sample in a closed container. (Ref: AOAC 17th edn, 2000, Official method 925. 08 Sampling of flour)

8.1. DETERMINATION OF MOISTURE

Weigh accurately about 5 gm of sample in a previously dried and tared dish and place the dish with its lid underneath in the oven maintained at 130 – 133°C for 2 hours. The time should be reckoned from the moment the oven attains 130°C after the dishes have been placed. Remove the dish after 2 hours, cool in the desiccators and weigh.

8.1.2 Calculation

Moisture (%) = $\frac{(W1- W2) \times 100}{W1- W}$

Where,

W1 = Weight in gm of the dish with the material before drying

W2 = Weight in gm of the dish with the material after drying

W = Weight in gm of the empty dish

8.2 DETERMINATION OF TOTAL ASH

Take fresh sample for the determination, rather than left over after determination of moisture. Ignite the dried material in the dish left after the determination of moisture with the flame of a burner till charred. Transfer to a muffle furnace maintained at 550 – 600°C and continue ignition till grey ash is obtained. Cool in a dessicator and weigh. Repeat the process of heating, cooling and weighing at half hour intervals till the difference in weight in two consecutive weighings is less than 1 mg. Note the lowest weight. 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.

Performance characteristics to be defined in the current method as proposed below:
Limit of detection : 0.055
Repeatability : 0.06gm/100gm
Reproducibility : 0.20 gm/100 gm for homogeneous products like milk powders

0.60 gm/100 gm for heterogeneous products like pet foods, meat products etc.

8.2.1 Safety and GLP aspects

Crucibles/Dishes must be cleaned carefully. Never use abrasive products such as sand, hot concentrated nitric acid, free alkalis or aqua regia.

Very hot crucibles/dishes must not come into contact with silica, quartz or metal oxides since there is a risk of alloy formation resulting in perforations. After use, wash the crucibles/dish with tap water, using a laboratory brush to remove any adhering material. Remove any stains with cold concentrated hydrochloric acid. In some cases it

may be necessary to melt potassium hydrogen sulphate ($KHSO_4$) in the crucible/dish. If this does not help, carefully rub the stain with wet Keiselguhr, talc or kaolin.

During the analysis do not touch crucibles/dish with fingers, but handle them with platinum-tipped tongs Maintain Internal control plan of the instruments, eg: Check actual temperature of the muffle furnace on a regular basis using a reference certified thermocouple. Re-calibrate temperature controller of muffle furnace if actual operating temperature is not within the range 550±25°C.

8.2.2 Calculation

Total ash on dry basis (% by weight) = $\frac{(W2 - W) X100}{W1 - W}$

Where,

W2 = Weight in gm of the dish with the ash

W = Weight in gm of empty dish

W1 = Weight in gm of the dish with the dried material taken for test

(Ref:- AOAC 17th edn Official method 923.03 Ash of flour)

*Moisture correction factor to be included in the formula

Reference of Method Recommended

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, (23.1.05).
3) AOAC International (1995) « Ash of milk – gravimetric method » in Official Methods of AOAC International, method 945.46, (33.2.10).
4) AOAC International (1995) « Ash of meat» in Official Methods of AOAC International, method 920.153, (39.1.09).
5) ISO 2171:1993 « Cereals and milled cereal products – Determination of total ash.
6) ISO/DIS 936 « Meat and meat products – determination of total ash » (Revision of ISO 936:1978).
7) AOAC 17th edn Official method 923.03 Ash of flour

8.3 DETERMINATION OF ASH INSOLUBLE IN DILUTE HCL

8.3.1 Reagents

a) Dilute HCl – Approx 5.5 N

Preparation of HCl: 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.

8.3.2 Procedure

To the ash contained in the dish, add 25 mL of dilute HCl, cover with a watch glass and heat on a water bath for 10 minutes. Allow to cool and filter the contents of the dish through Whatman filter paper No 42 or its equivalent. Wash the filter paper with water until the washings are free from acid and return it to the dish. Keep it in the electric oven for 3 hours to dry. Ignite in a muffle furnace at 550 – 600°C till white or grey ash is obtained. The filtrate can be dried for 30 min in an oven at 103±2°C. Cool in a dessicator and weigh. The filtrate obtained should be free for chloride/acid. To check this, add few drops of 2M nitric acid and 0.1 M Silver nitrate solution. No precipitate or milky turbidity should occur. Repeat the process of igniting, cooling and weighing till the difference in two consecutive weighs is less than 1 mg. Note the lowest weight.

Performance characteristics to be defined in the current method as proposed below:
Limit of detection: 0.05 %
Repeatability: not greater than 20%.

8.3.3 Safety and GLP aspects

Crucibles/Dishes must be cleaned carefully. Never use abrasive products such as sand, hot concentrated nitric acid, free alkalis or aqua regia.
Very hot crucibles/dishes must not come into contact with silica, quartz or metal oxides since there is a risk of alloy formation resulting in perforations.
After use, wash the crucibles/dish with tap water, using a laboratory brush to remove any adhering material. Remove any stains with cold concentrated hydrochloric acid. In some cases it may be necessary to melt potassium hydrogen sulphate ($KHSO_4$) in the crucible/dish. If this does not help, carefully rub the stain with wet Keiselguhr, talc or kaolin.

During the analysis do not touch crucibles/dish with fingers, but handle them with platinum-tipped tongs.

Maintain internal control plan of the instruments, eg: Check actual temperature of the muffle furnace on a regular basis using a reference certified thermocouple. Re-calibrate temperature controller of muffle furnace if actual operating temperature is not within the range 550±25°C.

8.3.4 Calculation

Ash insoluble in dilute HCl on dry wt basis = $\frac{(W2 - W) \times 100}{W1 - W}$

Where, W2 = weight in gm of dish with the acid insoluble ash

W =Weight in gm of empty dish

W1 =Weight in gm of the dish with the dried material.

8.4 DETERMINATION OF GLUTEN

8.4.1 Procedure

Weigh 25 gm sample into a dish and add about 15 mL of water to it and make it into a dough taking care that all the material is taken into the dough. Keep the dough gently in a beaker filled with water and let it stand for 1 hour. Remove the dough and place it in a piece of bolting silk cloth with an aperture of 0.16 mm (No, 10 XXX) and 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. 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, transfer to a watch glass or petri dish and keep it in the oven at 105±1°C for drying.

When partially dried, remove and cut into several pieces with a scissor and again keep in the oven to dry. Cool in a desiccators and weigh. Return it to the oven again for half hour, cool and weigh to ensure constant weight.

8.4.2 Calculation

Gluten on dry wt. basis = $\frac{\text{Wt of dry gluten} \times 100 \times 100}{\text{Exact wt. of sample} \times (100 - \text{Moisture content})}$

(Ref: - IS 1155 :1968 Method of Determination of Gluten)

8.5 DETERMINATION OF ALCOHOLIC ACIDITY

8.5.1 Reagents

(a) Neutral Ethyl alcohol – 90% (v/v)

(b) Standard Sodium Hydroxide solution – approx 0.05 N

(c) Phenolphthalein Indicator – Dissolve 0.1 gm in 100 mL of 60% Ethyl alcohol.

8.5.2 Procedure

Weigh 5 gm of sample in a stoppered conical flask and add 50 mL of neutral ethyl alcohol. Stopper, swirl gently and allow to stand for 24 hours with occasional swirling. Filter the alcoholic extract through a dry filter paper. Titrate10 mL of the alcoholic extract with standard sodium hydroxide solution to a pink end point using phenolphthalein as indicator. Subtract titre value of blank alcohol.

*Whatman filter paper No.1 or equivalent is to be used for filtration process

8.5.3 Calculation

Alcoholic acidity with 90 % alcohol calculated as H_2SO_4 on dry basis

$$= \frac{\text{Titre Value} \times 24.52 \times \text{Normality of NaOH}}{\text{Weight of sample (dry weight)}}$$

(Ref: - IS 12711 :1989 Method of Determination of Alcoholic Acidity)

8.6 DETERMINATION OF CALCIUM CARBONATE IN FORTIFIED ATTA

8.6.1 Principle

Calcium is precipitated as the insoluble oxalate from an ammonical solution, precipitate is dissolved in dilute sulphuric acid and titrated with Potassium permanganate until pink colour appears.

8.6.2 Procedure

Ash approximately 5 gm of sample at 550°C. Wash the ash into 250 mL beaker with 40 mL concentrated HCl and 60 mL water. Add 3 drops concentrated Nitric acid and boil for 30 minutes. Cool and transfer to a 250 mL volumetric flask. Make upto mark, mix and filter. Pipette a volume of the filtrate containing 10 – 40 mg of calcium in a 250 mL beaker. Add 1 mL of 30 % citric acid solution and 5 mL of 5% Ammonium

chloride solution. Make upto approximately 100 mL with water and bring to boil .Add 10 drops of bromo-cresol green solution (0.04%) and 30mL of warm saturated ammonium oxalate solution. If a precipitate forms dissolve it by adding a few drops of concentrated HCl. Neutralise very slowly with ammonia solution (0.88), stirring continuously until indicator changes colour at pH 4.4- 4.6. Place beaker on steam bath for 30 minutes. Remove the beaker and after 1 hour filter through a fine sintered glass crucible or No 42 filter paper. Thoroughly wash the beaker and crucible/filter paper and dissolve the ppt by passing through 50 mL of warm 10% sulphuric acid. Rinse the crucible/filter paper and make up the filtrate to about 100 mL with water. Heat the filtrate to 70–80^{0}C and titrate with 0.02 N Potassium permanganate solution until a pink colour persists.

2 mL of 0.02M (or 0.1N) potassium permanganate = 2.004 mg Ca

(Ref: - Pearson's Composition and Analysis of Foods 9th edn, page 33)

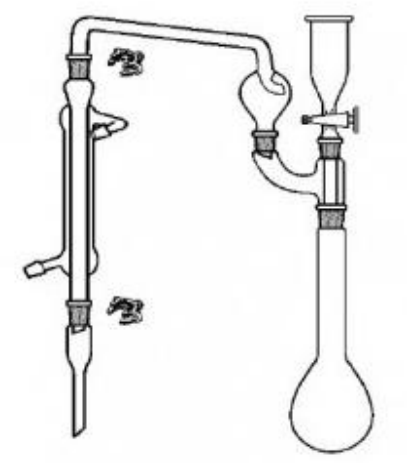
8. 7 DETERMINATION OF TOTAL PROTEIN IN PROTEIN RICH ATTA

8.7.1 Principle

The protein content is determined from the organic Nitrogen content by Kjeldahl method. The various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with an alkali (NaOH) and the ammonia liberated is absorbed in excess of standard solution of acid and then back titrated with standard alkali.

8.7.2 Apparatus

a. Kjeldahl digestion flask - 500 or 800 mL
b. Kjeldahl distillation apparatus, - same digestion flask fitted with rubber stopper through which passes lower end of efficient rubber bulb or trap to prevent mechanical carryover of NaOH during distillation or apparatus as shown below.
c. Conical flask, 250 mL
d. Burette 50 mL



Distillation apparatus

8.7.3 Reagents

a. Concentrated Sulphuric acid – Specifiic gravity 1.84
b. 45% Sodium Hydroxide - Dissolve 450 gm of Sodium hydroxide pellets in 1000 mL water
c. Standard Sulphuric acid solution – 0.1 N
d. Standard Sodium Hydroxide solution – 0.1 N
e. Methyl Red Indicator solution - Dissolve 0.5 gm methyl red in 100 mL of alcohol

8.7.4 Procedure

Weigh quickly about 1-2 gm of the sample and transfer to a 500 or 800 mL Kjeldahl flask taking care to see that no portion of the sample clings to the neck of the flask. Add 0.7 gm of Mercuric oxide, 15 gm of Potassium Sulphate and 40 mL of concentrated sulphuric acid (Mercuric oxide is added to increase the rate of organic breakdown during acid digestion. Because of environmental/safety concerns over handling and disposal of mercury, copper sulphate can be used. This is important from safety point of view as mercury vapours might escape into the environment during the distillation process. Also Missouri catalyst tablets known as Kjeldahl tablets (Composition: 48.8% Sodium sulphate & 48.9% Potassium sulphate & 0.3% copper sulphate) can also be used). Add two to three glass beads. Place the flask in an inclined position on the stand in the digestion chamber and digest. Heat the flask gently at low flame until the initial frothing ceases and the mixture boils steadily at a moderate rate. During heating rotate the flask several times. Continue heating for about an hour or

more until the colour of the digest is pale blue. If black specs are present after 30 minutes of digestion, wrap the vessel with aluminium foil and keep for 2-3 minutes. By doing this black specs would move down from the walls in the digestion mixture. If the specs are still present, remove the vessel from heat and allow to cool for 10 mins. Do not modify the heat intensity in the whole process. Alternatively, few drops of water may also be pour down across the side of the flask. Cool the digest and add slowly 200 ml of water. Cool, add a piece of granulated Zinc or anti bump granules and carefully pour down the side of the flask sufficient Sodium Hydroxide solution (450gm/L) to make the contents strongly alkaline (about 110 mL) before mixing the acid and alkaline layer.

Connect the flask to a distillation apparatus incorporating an efficient flash head and condenser. To the condenser fit a delivery tube which dips just below the surface of the pipetted volume of standard acid contained in a conical flask receiver. (Precaution: The receiving solution must remain below 45°C to prevent loss of ammonia). Mix the contents of the digestion flask and boil until 150 mL have distilled into the receiver. Add 5 drops of methyl red indicator and titrate with standardized 0.1 N Sodium Hydroxide solution.

Carry out a blank titration simultaneously.

1 mL of 0.1 N H_2SO_4 = 0.0014gm N

8.7.5 Calculation

Calculate protein as = N x 6.25

$$\text{Protein on dry wt. basis} = \frac{\text{Protein content} \times 100}{(100 - \text{Moisture content})}$$

Ideally the protein content of food stuff is calculated by multiplying its total nitrogen content by a factor 6.25. This factor is used whenever the nature of the protein is unknown or when the product to be analyzed is a mixture of different proteins with different factors. However use of different Nitrogen conversion factors for different matrices may lead to better accuracy of results.

Few examples:

6.38--for Milk and milk products (cheese/caseinate/whey and derivatives/yoghurt/lactose/ice-cream)

6.25--for Infant formula (hydrolyzed/partially hydrolyzed/follow-on)

6.25--for Infant cereals with milk (complete cereals, to be reconstituted with water)

5.8--for Infant cereals without milk (standard cereals, to be reconstituted with milk

(Ref: Pearson's Composition and Analysis of Foods, 9th edn, page 17)

8.7.6 Safety and GLP Aspects:

Perform digestion/distillation steps under well ventilated fume hood. Wear gloves and safety glasses when handling concentrated sulphuric acid and sodium hydroxide. Ensure neutralization of the Acid, before disposal. *Internal Control plan* - Ensure check of tightness of distillation apparatus and performance of titration. e.g. Before each distillation, check that the rubber bung of the distillation apparatus is perfectly clean and free from traces of salt. After each series of analysis remove it and rinse with water.

Each day of analysis, check the tightness of the distillation unit as follows: Pipette 10.0 mL of 0.05 mol/L ammonium sulfate solution (5.5) in a digestion vessel. Alkalize with NaOH (5.4). Distil and titrate. The volume of titrated 0.1 mol/L HCl (5.2) must be between 9.90 and 10.05 mL. Otherwise look for the source of error and eliminate it.

Precautions:

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.

8.7.7 Recommended:**Reference Methods**

a) ISO 8968-1/IDF 20-1:2001, Milk – Determination of nitrogen content – Part 1: Kjeldahl method.

b) AOAC 991.20 (2005), Nitrogen (total) in milk, Final action 1994, IDF-ISO-AOAC Method, Revised March 1996.

c) AOAC 979.09 (2005), Proteins in grains, Final action 1994.

d) AOAC 976.05 (2005), Protein (crude) in animal feed and pet food, Final action 1977.Codex- adopted AOAC Method, Revised March 1996.

8.7.8 Reference for conversion factor of Nitrogen to protein:

a) Official Journal of the European Union. Commission Directive 2006/141/EC of 22 December, 2006 on infant formulae and follow-on formulae.

b) Codex Alimentarius Commission. ALINORM 07/30/26. Appendix II: Draft revised standard for infant formula and formulas for special medical purposes m intended for infants.

c) Bulletin of the International Dairy Federation 405/2006. Comprehensive review of scientific literature pertaining to nitrogen protein conversion factors.

d) Greenfield, H and Southgate, D.A.T. (2003). Food composition data. 2nd edition. Rome: Food and Agriculture Organization of the United Nations (cited in NUTTAB 2006 Online version and NUTTAB 2006 Electronic release, Explanatory notes).

8.8 DETERMINATION OF CRUDE FIBER

8.8.1 Reagents*

(a) Dilute Sulphuric acid – 1.25% (w/v) accurately prepared

(b) Sodium Hydroxide solution - 1.25% (w/v) accurately prepared

(c) Ethyl alcohol – 95% by volume

(d) Petroleum ether

*Reagents/chemicals used may be of AR grade.

8.8.2 Procedure

Weigh accurately about 2.5-3 gm 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. Transfer the whole of the boiling acid to the flask containing the defatted material and immediately connect the flask with a water cooled reflux condenser and heat so that the contents of the flask begin to boil within 1 minute. Since there is a risk of foaming and bumping, add a few drops of octanol after addition of sulphuric acid, to prevent foaming and boiling/glass beads in the flask, to prevent bumping. Rotate the flask frequently taking care to keep the material from remaining on the sides of the flask and out of contact with the acid.

Continue boiling for exactly 30 minutes. Remove the flask and filter through fine linen (about 18 threads to a 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). Filter aids can be added for better filtration and recovery of the analyte (filter aid Celite (R) 545). 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. Immediately connect the flask to the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the filtering cloth. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible prepared with a thin compact layer of ignited asbestos. Wash the residue thoroughly first with hot water and then with about 15 mL of ethyl alcohol. Dry the Gooch crucible and contents at 105±2°C in an air oven until constant weight is achieved. Cool and weigh. Incinerate the contents of the Gooch crucible in a muffle furnace until all carbonaceous matter is burnt. 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 hours).

Performance characteristics to be defined in the current method as proposed below:

- Limit of detection of approx 0.2gm/100gm crude fibre in the product.
- Repeatability limit of 0.3 gm/100 gm when the crude fibre content is less than 10 gm/100 gm product and 3% of the average when the crude fibre content is equal to or greater than 10 gm/100 gm product.

It is recommended to use fume-hoods for handling Sulphuric acid, Sodium hydroxide etc. Also ensure neutralization of the acid/base used prior to disposal. Dilute the concentrated sulphuric acid first. Internal Control plan of the instruments to be maintained (eg: soxhlet extractor). Against use of asbestos it is recommended to use filter aid Celite (R) 545, 22140 Fluka.

8.8.3 Calculation

$$\text{Crude fiber \% by wt} = \frac{(W1 - W2) \times 100}{W}$$

Where,

W1 = wt in gm of Gooch crucible and contents before ashing

W2 = wt in gm of Gooch crucible containing asbestos and ash

W = wt in gm of the dried material taken for the test

Calculate crude fibre on dry wt. basis by giving correction for the moisture content.

(Ref: Official Journal of the European Communities N° L 344/36 of 26.11.92, Determination of crude fibre/ AOAC, 2005, 962.09)

9.0 MAIDA, FORTIFIED MAIDA, PROTEIN RICH MAIDA

Follow methods of analysis described for Atta, fortified atta and protein rich atta (8.1-8.5)

9.1 Determination of Thiamine in Fortified Maida

9.1.1 Principle

Bound thiamine of foods is released by incubation with Takadiastase and papain at acidic pH. The crude extract is purified by passing through a column of activated Decalso (or its equivalent). Thiamine in the eluate is estimated by oxidizing it to Thiochrome and measuring the fluorescence. Lead acetate may be used if Decalso (or its equivalent) is not available.

9.1.2 Apparatus

a. Chromatographic Columns - 25 × 1 cm.
b. Centrifuge
c. Spectrofluorimeter
d. Waring Blender

9.1.3 Reagents

a) Sodium Acetate Buffer (0.20 M, pH 4.2)- . Dissolve 34 gm of sodium acetate ($CH_3COONa\ 3H_20$) in 250 mL of water. To 30 mL of this solution, add 70 mL of IN acetic acid and dilute to 500 mL.
b) Enzyme Solution - Suspend 150 mg of Takadiastase and 75 mg of papain in 5 mL of acetate buffer.
c) Stock Thiamine Solution-Dissolve 25 mg of pure dry crystalline thiamine hydrochloride in 250 mL of 0.01 N hydrochloric acid (1 mL = 100 µg).

d) Acetic Acid - 3 % (v/v).

e) Acetic Acid - 0.5 %t (v/v).

f) Activated Decalso or Equivalent - Wash Decalso (or its equivalent) which passes through 180- to 250-µ IS Sieve (see IS: 460-1962) successively once with hot 3 % acetic acid, once with hot potassium chloride solution (g) and again with hot 3 % acetic acid followed by several washes with hot distilled water. Each washing consists of stirring Decalso in the liquid for 15 minutes, allowing it to settle and then decanting. The final wash solution should be free of chlorides when tested with 1 % silver nitrate solution. Store the Decalso under water in a stoppered bottle.

g) Potassium Chloride Solution - 25% (w/v) in 0.1N hydrochloric acid .

h) Bromocresol Green Indicator - 0.4% in 70% ethyl alcohol.

i) Isobutyl Alcohol - Redistilled (in an all glass apparatus), fraction distilling at 105-108°C collected and saturated with water.

j) Anhydrous Sodium Sulphate

k) Potassium Hydroxide Solution - 15% in water (w/v).

l) Potassium Ferricyanide Solution - 1% in water (w/v)

m) Oxidising reagent - Prepared fresh before use. Mix 1 part of potassium ferricyanide solution (l) with 9 parts of potassium hydroxide solution (k) .

n) Blank reagent - Mix 1 part of water with 9 parts of potassium hydroxide (k) solution.

o) Basic Lead Acetate Solution - Dissolve 180 gm of Lead acetate in about 700 mL of distilled water by heating heat. To the hot solution add 110 gm of finely powdered lead-oxide (litharge) and continue boiling for 45 minutes with constant stirring. Cool the mixture, filter and dilute to 1 litre.

9.1.4 Extraction

Mix the atta/maida thoroughly until homogeneity is achieved. Homogenize the flour well in a blender with a suitable volume of water. Weigh the total slurry and use a weighed aliquot (equivalent to 10 to 20 gm of flour) for extraction of thiamine

Take two aliquots of the slurry or powder into 250-mL flasks marked 'Test' and 'Recovery ' and add 5 mL of enzyme suspension and 80 mL of sodium acetate buffer to both. To the flask marked 'Recovery' add diluted thiamine standard solution equivalent to 25 µg of thiamine hydrochloride (30 µg if using Lead acetate for purification). Add 2

to 3 mL of toluene to both the flasks and incubate at 37°C overnight (12-14h). Inactivate the enzymes by heating in a boiling water-bath, make up the volume to 100 mL and centrifuge or filter to remove the residue.

9.1.5 Purification

Two methods have been described 9.1.5.1 using Decalso and 9.1.5.2 may be used if Decalso is unavailable.

9.1.5.1 Decalso Method

Place small pieces of glass wool at the bottom of two chromatography columns (25 × 1 cm) (marked 'Test' and 'Recovery') and fill them uniformly with activated Decalso, to a height of 8 cm. Wash the columns with 3 mL of 0.5 % acetic acid. Take 20 mL each of test and recovery samples in beakers and adjust them to pH to 3.5 with 1 N hydrochloric acid. The end point may he checked in pH meter or with Bromocresol green indicator (yellowish green colour). Pour the samples and the washings on the respective columns and allow the liquid to drain. Wash the columns two times with 10 mL portions of boiling water and discard the elutes. Eluate thiamine from the columns successively with 10, 10 and 5 mL of boiling potassium chloride solution and collect directly in a 25-mL volumetric flask or in a conical flask and make up the volumes to 25 mL after cooling.

9.1.5.2 Lead acetate method

To 20 mL of the food extract, add 10 mL of the basic lead acetate solution, mix and centrifuge. To 20 mL of the supernatant, add 3 mL of 30 % (v/v) sulphuric acid and 17 mL of water. Remove the precipitate by centrifugation and store the supernatant solution at 5°C.

9.1.6 Procedure

Conversion to Thiochrome-Take two 5-mL aliquots (10 mL in the case of extracts obtained with the lead acetate method) from the purified extract marked 'Test' into two separatory funnels (50 mL) or glass-stoppered test-tubes labeled 'Test' and 'Blank'. In a third tube labeled 'Recovery' take 5 mL of the purified extract marked 'Recovery'(10 mL in case of extracts obtained with the lead acetate method). To the ' Blank' add 1 mL of the blank reagent, followed within 1 minute by 15 mL of isobutyl alcohol. To the 'Test'

and 'Recovery' tubes (or separatory funnels) add 1 mL of the oxidizing reagent, followed by 15 mL of isobutyl alcohol. Shake both the tubes (separatory funnels) vigorously for 1 minute, allow the layers to separate and transfer the upper butanol layer containing thiochrome to another test-tube (use a 10-mL pipet with a rubber bulb to separate the epiphase if separatory funnels are not used). Add a small quantity of anhydrous sodium sulphate to remove the traces of water and read the fluorescence of the clear extracts in a fluorometer using suitable filters for thiamine estimation or measure the fluorescence in a spectrofluorimeter (Excitation 365 nm and Emission at 435 nm) A suitable reagent blank and a standard containing 2-5 µg of thiamine hydrochloride should also be prepared to balance the fluorimeter.

9.1.7 Calculation

Calculate µg of thiamine per gm of food sample (on dry basis or wet basis as the case may be) as follows

$$\frac{\text{Test Reading- Blank Reading}}{\text{Recovery Reading- Test Reading}} \times \frac{\text{Dilution Factor}}{\text{Weight of substance taken (or present in the slurry)}}$$

The dilution factor in the Decalso method would be 25 and in the Lead acetate method it would be 30.

9.1.8 Reference

Methods For Estimation Of Thiamine (Vitamin B1) In Foodstuffs (IS : 5398 - 1969, Reaffirmed 2005)

AOAC Official Method 953.17 Thiamine (Vitamin B1) in grain products, Fluorimetric (Rapid) Method

9.2 Determination of Riboflavin in Fortified Maida

9.2.1 Principle

Riboflavin fluoresces when exposed to light of wave- length 440 to 500 nm. The intensity of the fluorescence is proportional to the concentration of riboflavin in dilute solutions. Riboflavin phosphate (FMN) and flavin adenine dinucleotide (FAD) exhibit the same characteristic yellow colour and yellow green fluorescence as riboflavin. The

riboflavin is measured in terms of difference between the fluorescence before and after chemical reduction by hydrosulphite which will reduce riboflavin and its co-enzymes to colourless compounds which do not fluoresce

9.2.2 Apparatus

Spectrofluorimeter or Fluorimeter having an input filter of narrow transmittance with maximum of about 440 nm and the output filter of having transmittance with a maximum of about 565 nm.

9.2.3 Reagents

a) Standard Hydrochloric Acid - O-1 N.
b) Sodium Hydroxide Solution - 4 % (w/v).
c) Dilute Hydrochloric Acid - 1:l (v/v).
d) Riboflavin Stock Solution I - Add 50 mg of USP riboflavin reference standard or equivalent IP standard previously dried and stored in dark in a desiccator over phosphorus pentoxide to about 300 mL of 0.02 N acetic acid and warm the mixture on a steam-bath with constant stirring until the riboflavin is completely dissolved, cool and then add 0.02 N acetic acid to make the volume to 500 mL. Store the solution under toluene in the cold in a dark bottle. One mL of this solution is equivalent to 100 µg of riboflavin.
e) Riboflavin Stock Solution II - To 50 mL of the riboflavin stock solution I, add 0.02 N acetic acid solution to make 500 mL. Store the solution under toluene in the cold in a dark bottle. One mL of this solution is equivalent to 10 µg of riboflavin.
f) Standard Riboflavin Solution-Dilute 10 mL of the riboflavin stock solution II with water to make 100 mL. One mL of this solution is equivalent to 1 µg of riboflavin. Prepare this solution fresh for each assay.
g) Glacial Acetic Acid
h) Potassium Permanganate Solution-Dissolve 4 gm of potassium permanganate crystals in 100 mL of water, keep for a few days, filter and store in a dark brown bottle.
i) Hydrogen Peroxide Solution - 3%.
j) Sodium Hydrosulphite -of high purity, unexposed to light or air.

9.2.4 Procedure

Precaution *Throughout the procedure, keep the pH of the solution below 7-O to prevent loss of riboflavin.*

9.2.4.1 Preparation of Sample Solution - Take a weighed quantity of flour in a flask of suitable size and add to it a quantity of the standard hydrochloric acid equal in millilitres to not less than 10 times the dry weight of the sample in grams so that the resulting solution shall not contain more than 0.1 mg/mL of riboflavin . If the material is not readily soluble, then comminute the sample so that it may be evenly dispersed in liquid. Then agitate the solution vigorously and wash down the sides of the flask with the standard hydrochloric acid. Heat the mixture in an autoclave at 121-123°C for 30 minutes and cool to 25±2°C. If lumping occurs, agitate the mixture until the particles are evenly dispersed. Adjust with vigorous agitation the pH of the mixture to 6.0 to 6.5 with the sodium hydroxide solution. Add dilute hydrochloric acid immediately until no further precipitation occurs. Dilute the mixture to a known volume such that final mixture contains more than 0.1 µg /mL riboflavin. Filter the mixture through a filter paper (Whatman No. 1 or equivalent) which does not absorb riboflavin. In case the mixture is difficult to filter, centrifuge it and filter through fritted glass, using suitable analytical filter-aid which may often be substituted for, or preceded by filtering through a filter paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding drop wise, first dilute hydrochloric acid and if no precipitate forms, then the sodium hydroxide solution with vigorous agitation and proceed as follows:

a) If no further precipitation occurs, adjust the pH to 6.8 using sodium hydroxide solution with vigorous agitation. Dilute the solution to contain ~0.10 µg/mL of riboflavin. If cloudiness appears, filter again.
b) If further precipitation occurs, adjust the solution again to the point at which the maximum precipitation occurs. Dilute the solution such that it contains more than 0.10 µg/mL of riboflavin. Filter and take the aliquot of the clear filtrate and proceed further as described above.

9.2.5 Estimation

Add 10 mL of the sample solution in two tubes. To one of these tubes, add one mL of the standard riboflavin solution and mix and to other add one mL of water and mix. Add 1 mL of acetic acid to both the tubes and mix. The add with mixing, 0.5 mL of potassium

permanganate solution (quantity may be increased for sample solutions that contain excess of oxidizable material but not more than 0.5 mL in excess of that required for complete oxidation of foreign matter should be added). Allow to stand for 2 minutes. Then add with mixing O-5 mL of the hydrogen peroxide solution (the permanganate colour gets destroyed within 10 seconds). Shake the tubes vigorously to expel all excess oxygen. If gas bubbles remain on the sides of the tubes after the foaming ceases, remove by tipping the tubes so that the solution flows slowly from end to end.

NOTE -In case of frothing, a drop of alcohol, acetone or n-octanol may be added.

Measure fluorescence of the sample solution containing the added one mL of the standard riboflavin solution (Reading A). Measure fluorescence of the sample solution containing 1 mL of added water (Reading B). Then add with mixing, 20 mg of powdered sodium hydrosulphite to this tube (see Note). Measure fluorescence within 5 seconds (Reading C).

NOTE-The sodium hydrosulphite shall be of high purity and kept from undue exposure to light or air. A quantity appreciably in excess of 20 mg may reduce foreign pigments and fluorescing substances or both, thereby causing erroneous results.

Suitability of the sodium hydrosulphite may be checked as follows : To each of two or more tubes, add 10 mL of water and 1 mL of the standard riboflavin solution containing 20 µg/mL of riboflavin and proceed by addition of acetic acid, potassium permanganate solution and hydrogen peroxide solution. Then, upon addition with mixing of 8 mg of sodium hydrosulphite, the riboflavin should be completely reduced in not more than 5 seconds.

9.2.6 Calculation

Calculate mg of Riboflavin content of the samples on the basis of aliquots taken as follows:

$$\text{mg of riboflavin/ml of the final sample solution} = \frac{B-C}{A-B} \times \frac{1}{10} \times \frac{1}{1000}$$

The value of $\frac{B-C}{A-B}$

Shall not be less than 0.66 and should not exceed 1.5

Express the results as riboflavin mg/lOOgm (on dry basis or wet basis, as the case may be). Express the results as riboflavin mg/lOOgm (on dry basis or wet basis, as the case may be).

9.2.7 Reference

Methods For Estimation Of Riboflavin (Vitamin B2) In Foodstuffs (IS : 5399 - 1969, Reaffirmed 2010)

AOAC Official Method 970.65, Riboflavin (vitamin B2) in foods and vitamin preparations. Fluorimetric method, Final Action 1971

10.0 SEMOLINA (SUJI)

10.1 Preparation of sample for determination of Gluten

Grind the sample using a pestle and mortar or in a suitable grinding mill to a fine powder to pass through I.S 150 micron sieve, (aperture size 0.16 mm). Use this prepared sample for determination of gluten. For the remaining tests follow methods of analysis prescribed for Atta (clause 8).

11.0 DETECTION OF KESARI DAL POWDER (*Lathyrus sativus*) IN BESAN

11.1 Principle

The presence of kesari dal powder is detected on the basis of the presence of an unusual amino acid namely Beta-N-oxalyl L amino alanine (BOAA), which is not present in the seeds of other legumes

11.2 Apparatus

(a) Steam bath/water bath
(b) Air Oven
(c) Chromatographic paper Whatman No. 1 or equivalent

11.3 Regents

(a) Ethyl alcohol: 70%
(b) Isopropanol solution: 10%
(c) Liquified Distilled Phenol- water solution - (4: 1)
(d) Ninhydrin solution – 0.1% in acetone or ethanol

(e) Buffer – Pyridine : Acetic acid : water (0.5: 5 : 95) pH 3.60

11.4 Procedure

Weigh approximately 5 gm powdered sample and extract it with100 mL ethyl alcohol (70%) by keeping overnight, with occasional shaking. Filter the extract and evaporate to dryness on a steam/water bath. Extract the residue with 10 mL of iso-propanol solution, filter and use this solution for chromatography. Spot 20 µL of the extract using a haemoglobin pipette or capillary tube at a distance of 1 cm from the bottom of the chromatographic filter paper. Develop in a solvent chamber saturated with phenol–water solution overnight. Remove from the chamber, chromatogram in a current of air at room temperature for 4-5 hours or in an oven at 80°C for 1 hour and spray with ninhydrin solution. Dry the chromatogram in the oven for 15 minutes. The appearance of bluish – purple spot at about R_f value 0.1 shows presence of BOAA which is present only in *Lathyrus sativus*. Other proteins extracted simultaneously also give similar colour but at different R_f values. Always run known sample of kesari dal powder simultaneously and compare the spot of control with sample under test.

(Ref: ISI Handbook of Food Analysis (Part IV) – 1984 Page 121)

Note: Suitable precautions while drying the chromatograph shall be taken to avoid ingestion of hazardous vapours.

11.5 Detection of Kesari Dal Powder (*Lathyrus sativus)* in Besan by capillary electrophoresis method

Method 1

11.5.1 Principle

The presence of kesari dal powder is detected on the basis of the presence of an unusual amino acid namely Beta – N – oxalyl L amino alanine (BOAA), which is not present in the seeds of other legumes.

11.5.2 Apparatus

a) Centrifuge

b) Capillary Electrophoresis HPLC System- CZE system

c) Filter

d) Filter paper

11.5.3 Reagents

a) Ethanol

b) Ultra distilled water

c) Sodium hydroxide

11.5.4 Procedure

Weigh approximately 0.5 gm of *L. sativus* seeds. Soak in 50 mL ethanol-water (30:70, v/v) solution and shake for 2 hours (in ice). After centrifugation (3500 rpm at 15 minutes), filter the upper clear solution with 0.45 µm filter paper. Dilute the clear solution with ultra distilled water (1:1) and inject directly into the CZE (capillary zone electrophoresis) system for 40s at 50m bar. Capillary lenght 55 cm (44 cm effective length) × 50m. Perform the analyses at a constant voltage of 20 kV at 20°C in an electrolyte of 75 mM Borate buffer at pH 7.5. Adjust the required pH of the buffer by adding Sodium hydroxide. Use all chemicals of analytical-reagent grade. Determine the BOAA using capillary zone electrophoresis (CZE- modified -Zhao et al., 1999) and UV detection at 195 nm.

(Ref: UgurBasaranet al. African Journal of Biotechnology Vol. 10(20), pp. 4072-4080, 16 May, 2011).

Method 2

11.5.1 Apparatus

a) Capillary electrophoresis instrument equipped with a diode array detector and to operate via chemstation software.

b) Capillary of uncoated fused silica with a dimension of 48.5 cm × 50µm and length of 40cm.

11.5.2 Reagents

a) Double Glass distilled water

b) AR grade Beta – N – oxalyl L amino alanine (BOAA)

c) o-Phthalaldehyde (OPA) reagent: Prepared fresh. 100 mg of OPA, 200 µL of mercaptoethanol, 1 mL of absolute ethanol, 99 mL of 0.05 M SSodium borate buffer, pH 9.9.

d) Spectrophotometry. Seed extracts were analyzed for α-and 1)-ODAP according to the spectrophotometric method of Rao (1974) as modified by C. C. Campbell (1993. Agriculture Canada, personal communication), for each sample solutions.

11.5.3 Procedure

Prepare the hydrolysates by boiling 2 mL of seed extract in 4 mL. of 3M Potassium hydroxide for 30 minutes. Centrifuge the hydrolysates Pipette 0.25 mL in to 0.75 mL of water. Add 2 mL of o-phthalaldehyde (OPA) reagent Add 2 mL of 0.05 M Sodium borate buffer pH 9.9 for the A4 solution. Prepare another pair of tubes with unhydrolyzed extract to correct for any background absorbance (A1 with OPA reagent, A2 without OPA reagent). Keep the solution at 40°C for 2 hours and read the absorbance at 425 nm. Analyse ny Capillary electrophoresis UV detection at 195 nm. The analyses were performed at a constant voltage of 25kV at 40°C in an electrolyte of 20 mM Sodium phosphate (Na_2HPO_4) buffer at pH 7.8. Condition the capillary prior to each run by flushing it with 0.1 M NaOH for 2 min and with the electrolyte for 3 min. The electrolyte is replenished every third run. Inject the filtered seed extracts for 4-12s at 25m bar depending on the concentration of the extract. Ascribe the formula $(A_3 - A_4) - (A_1 – A_2)/3$ for the calculation of the absorbance change for BOAA. Use A standard curve based on dilutions of a Diamino propionate (DAP) solution (2.89 mM in ethanol-water, 6:4) for the quantification purpose.

(Ref: Arenfot et al. J. Agric. Food Chem., Vol. 43, No. 4, 1995).

12.0 DETERMINATION OF TALC IN RICE AND PULSES

12.1 Principle

The talc is floated off, filtered, digested, ignited and weighed**.**

12.2 Reagents

(a) 10 % Ammonia solution
(b) 3 % Hydrogen peroxide
(c) Hydrochloric- chromic acid mixture – Carefully dissolve 10 gm of Chromium Trioxide in 100 mL of water and add to 900 mL of concentrated Hydrochloric acid

12.3 Procedure

Shake 20 gm of sample with the ammonia (10%) and hydrogen peroxide (3%)

solutions. Heat to about 60°C so that the gas formed causes the particles of talc to come away from the surface. Decant off the liquid containing talc, wash the grains several times with water and add these washings to the decanted liquid. Heat the liquor with the Hydrochloric- Chromic acid mixture to oxidize suspended meal, filter off the talc, wash, ignite and weigh.

In unpolished rice this talc residue does not normally exceed 0.025%.

(Ref: - FAO Manuals of Food Quality Control 14/8, page 200)

13.0 MICROSCOPIC STRUCTURE OF CEREAL STARCHES

13.1 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

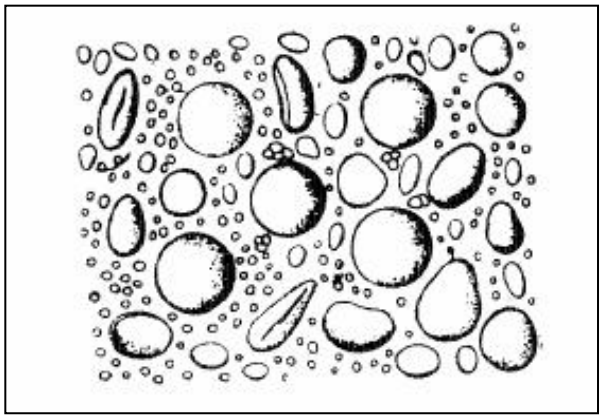
13.2 Procedure

Take a small quantity of the sample (1 gm or less) in a test tube or beaker and add about 50 mL water. Stir the contents with the help of glass rod to break up granules and lumps if any. Let it stand for a few minutes. 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. Remove excess liquid on the slide with a piece of blotting paper. Examine the slide under the microscope.

(Ref: - IS:4706 (Part I) 1978 Methods of test for edible starches and starch products)

13.3 Wheat starch

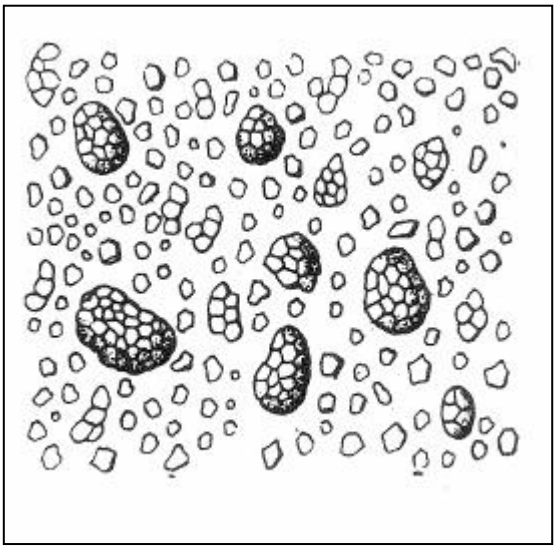
The small grains vary from 2 μm to 8 μm in diameter averaging about 6 -7 μm They are rounded or oval in outline, seldom polygonal or pointed. The large grains in surface view appear sometime rounded, sometime slightly irregular or oval but when touching the cover slip with the needle they are made to 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.



Wheat Starch

13.4 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.



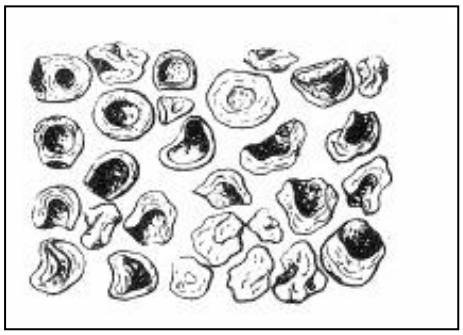
Rice Starch

13.5 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.

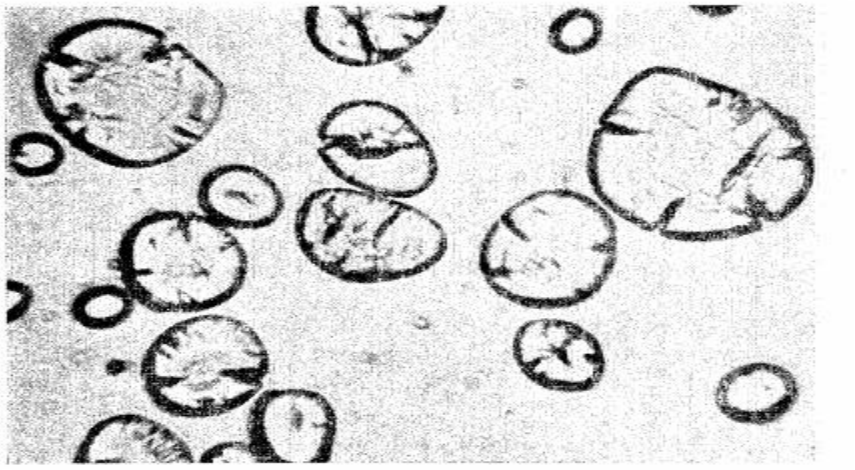
The granules of Tapioca soften when soaked in water for a few hours and preserve their original shape and exhibit a distinct hilum. In many the hilum is stellately fissured, in others the central part of the grain is a translucent mass but the outline is still recognizable, whilst finally many have swollen 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



Tapioca Starch

13.6 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.



Arrowroot starch

(Ref: - FAO Manuals of Food Quality Control, 14/8, pages 204 – 215)

14.0 BISCUITS

14.1 Types of Biscuits

Biscuits come in different varieties depending on their sensory qualities. They are classified as Sweet, Semisweet, Crackers, Cookies and speciality biscuits.

14.2 Preparation of sample

Biscuits are highly hygroscopic and take up moisture quickly when exposed to atmosphere. The preparation of sample should be done very quickly preferably in a dry place. Grind the sample as quickly as possible after removing the cream, caramel, chocolate, marshmallow, jam, jelly, or any other filling between the biscuits etc. The coating if any should also be removed by gentle scraping before powdering the sample. Store the powdered material in a dry airtight glass container.

(Ref: - IS 12741 – 1989 Bakery products – Methods of sampling)

14.3 Determination of moisture

14.3.1 Apparatus

a) Moisture dish – Porcelain, silica, glass or Aluminium (7.5 × 2.5 cm.)

b) Oven: electric maintained at 105±10°C

c) Desiccator

14.3.2 Procedure

Weigh accurately about 5gm of the powdered sample in the moisture dish previously dried in an oven and weighed. Place the dish in the oven maintained at 105±10°C for four hours. Cool in the desiccator and weigh. Repeat the process of drying, cooling, and weighing at 30 minute intervals until the difference in two consecutive weighs is less than 1 mg. Record the lowest weight.

14.3.3 Calculation:

$$\text{Moisture \% by weight} = \frac{100(W1-W2)}{W1 - W}$$

Where,

W1 = weight in gms of the dish with material before drying.

W2 = weight in gms of dish with material after drying to constant weight.

W = weight in gms of the empty dish

(Ref: - IS 1011 – 1992 Biscuits – Specification)

14.4 Determination of Acid Insoluble ash

14.4.1Apparatus

(a) Dish - silica or porcelain - platinum dish should be preferred.

(b) Muffle furnace – maintained at 600±20°C

(c) Water bath

(d) Desiccator

14.4.2 Reagents

Dilute Hydrochloric acid – approx. 5 N, or 1:2.5 HCl prepared from concentrated hydrochloric acid.

14.4.3 Procedure

Weigh accurately about 20 gm of the biscuit powder in the dish and incinerate first on hot plate and then ash in the muffle furnace at 550±20°C until light grey ash is obtained.

Remove the dish from the furnace and allow it to cool at room temperature. Add 25 mL of the hydrochloric acid to the dish, cover with a watch-glass and heat on the water-bath for 10 minutes. Mix the contents with the tip of a glass rod and filter through Whatman filter paper no. 42 or its equivalent. Wash the filter paper with water until the washings are free from acid when tested with blue litmus paper Return the washed filter paper to the dish for ashing in the muffle furnace as above. Cool the dish in a desiccator and weigh. Again ignite the dish for half an hour in the furnace, cool and weight. Repeat this operation until the difference between successive weighings is less than 1 mg. Filter 25 mL of the hydrochloric acid through a blank filter paper, wash, ash and weigh it as in the case of acid insoluble ash. Subtract its weight from the weight of the insoluble ash of the sample.

14.4.4 Calculation

Acid insoluble ash, % by weight $A = \frac{100\ (W1-W)}{W2}$

Where,

W1 = weight in gm of the dish containing acid insoluble ash.

W = weight in gm of the empty dish in which the sample is taken for ashing,

W2 = weight in gm of the sample.

Note: Correct the acid insoluble ash weight for the blank of filter paper, if any.

Acid insoluble ash, % by $= \frac{(A \times 100)}{100-M}$ (dry basis)

Where,

A = acid insoluble ash, % by weight, and

M = percentage of moisture in the biscuit.

(Ref: - IS 1011: 1992 Biscuits – Specification)

14.5 Determination of acidity of extracted fat

14.5.1 Apparatus

Soxhlet Apparatus – with a 250 mL flat bottom flask.

14.5.2 Reagents

(a) Petroleum Ether – Boiling point 40 to 80°C

(b) Benzene- Alcohol–Phenophthalein Stock solution – To one L of distilled benzene add one L of alcohol or rectified sprit and 0.4 gm of phenophthalein. Mix the contents well.

(c) Standard sodium hydroxide solution – 0.05N

14.5.3 Procedure

Weigh accurately sufficient amount. of biscuit powder (20 – 25 gm) which will yield 3- 4 gm of fat and transfer it to a thimble and plug it from the top with extracted cotton and filter paper. In case of filled and coated biscuits the weight of the biscuits includes the filling and coating material. Dry the thimble with the contents for 15 to 30 minutes at 100°C in an oven. Take the weight of empty dry soxhlet flask. Extract the fat in the soxhlet apparatus for 3 to 4 hours and evaporate off the solvent in the flask on a water bath. Remove traces of the residual solvent by keeping the flask in a hot air oven for about 30 minutes. Cool the flask. Weigh accurately about 3.0 gm of extracted fat in a 250 mL conical flask and add 50 mL of mixed benzene-alcohol-phenolphthalein reagent and titrate the contents to a distinct pink color with the Sodium hydroxide solution taken in a 10 mL micro burette. If the contents of the flask appear cloudy during titration add another 50 mL of the reagent titration. Make a blank titration with 50 mL of the reagent.

Subtract from the titer of the fat, the blank titer.

14.5.4 Calculation

Acidity of extracted fat (as oleic acid) % by weight = $\frac{28.2 \times V \times N}{W1-W}$

Where,

V = Volume of 0.05N Sodium hydroxide solution used in titration after subtracting the blank

N= Normality of Sodium hydroxide (determined using Potassium phthalate)

W1 = weight in gm of soxhlet flask containing fat

W = weight in gm of the empty soxhlet flask

(Ref: - IS 1011: 1992 Biscuits – Specification)

15.0 BREAD

15.1 Preparation of sample

Cut the sample into small pieces and mix together so as to form a composite sample and transfer to a clean dry airtight glass container.

(Ref: - IS 12711:1989 Bakery products – Methods of Analysis)

15.2 Determination of Moisture

15.2.1 Apparatus

(a) Moisture dish – made of porcelain, silica, aluminium, stainless steel (7.5x 2.5 cm)
(b) Oven, electrical maintained at 105±2°C
(c) Desiccators

15.2.2 Procedure

Weigh accurately about 5 gm of the prepared sample in a moisture dish previously dried in the oven at 105^0C. Place the dish in the oven maintained at 105±2^0C for 4 hours. Cool in a desiccator and weigh. Repeat this process of heating, cooling, weighing till the difference between two consecutive weighing is less than 1 mg. From the loss in mass, calculate the percentage of moisture.

15.2.3Calculation

Moisture % by weight = $\frac{100(W_1-W_2)}{(W_1-W)}$

W1 = weight in gm. of dish with material before drying.
W2 = weight in gm. of dish with material after drying to constant weight.
W = weight in gm. of empty dish.

15.3. Determination of Alcoholic acidity

15.3.1 Reagents

(a) Neutral Alcohol 90 % (v/v)
(b) Standard NaOH solution – 0.05 N
(c) Phenolphthalein solution – 1% solution in ethyl alcohol

15.3.2 Procedure

Weigh about 5.0 gm of dried sample into a stoppered conical flask and add 50 mL of 90% neutral alcohol, previously neutralized against phenolphthalein. Stopper, shake and allow to stand for 24 hours, with occasional shaking. Filter the alcoholic extract, through a dry filter paper. Titrate the combined alcoholic extract against 0.05 N standard sodium hydroxide solution using Phenolphthalein as an indicator.

15.3.3 Calculation

No. of mL of 1N NaOH required for neutralization of 100 gm of sample

$$= \frac{\text{Titer} \times \text{Normality of NaOH} \times 100}{\text{Wt. of the sample taken}}$$

(Ref: - IS 12711: 1989 Bakery Products – Methods of Analysis)

15.4 Determination of Acid Insoluble Ash

15.4.1 Apparatus

(a) Platinum dish or Silica dish
(b) Water bath
(c) Dessicator
(d) Muffle Furnace

15.4.2 Reagent

(a) Dilute hydrochloric acid (5N) or 1:2.5 HCl prepared from concentrated hydrochloric acid.

15.4.3 Procedure

Weigh accurately about 5 gm of the prepared sample in a tared clean and dry dish. Ignite the material in the dish with the flame of suitable burner for about an hour. Complete the ignition by keeping in a muffle furnace a temperature between 550±10°C until grey ash is obtained. Cool in the desiccator and weigh. Repeat the process of igniting, cooling and weighing at one hour interval until the difference between two successive weighs is less than 1 mg.

To the ash contained in the dish, add 25 mL. of dilute hydrochloric acid, cover with a watch-glass and heat on a water bath for 10 minutes. Allow to cool and filter the

contents of the dish through Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid. Return the filter paper and residue to the dish. Keep it in an electric air oven and heat till it gets dried. Ignite the contents of the dish over a burner till it gets completely charred. Complete the ignition by transferring the dish to the muffle furnace maintained at 550±100°C until grey ash is obtained. Cool the dish in desiccator and weigh. Heat the dish again in the furnace for 30 minutes.

Cool in the dessicator and weigh. Repeat the process of heating for 30 minutes, cooling, weighing until the difference between the two successive weighing is less than one milligram. Record the lowest mass.

15.4.4 Calculation

Acid insoluble ash, percentage by weight $A = \frac{100\ (W1-W)}{W2}$

Where,

W1 = weight in gm of the dish containing acid insoluble ash.

W = weight in gm of the empty dish in which the sample is taken for ashing

W2 = weight in gm of the sample

Note: Correct the acid insoluble ash weight for the blank of filter paper, if any

Acid insoluble ash, % by weight (on dry basis) $= \frac{(A \times 100)}{100-M}$

Where,

A = acid insoluble ash, percentage by weight, and

M = percentage of moisture in the bread

(Ref: - IS 12711: 1989 Bakery Products – Methods of Analysis)

15.5 Determination of Non –Fat Milk Solids in Milk Bread

15.5.1 Principle

The method is a colorimetric one for estimation of non fat milk solids in bread

based on the orotic acid (2, 6 dihydroxy pyrimidine- 4 carboxylic acid) content. The mean orotic acid content of non fat milk solids is 62.5 mg/100 gm (range 48.0 – 74.5 mg/100 gm).

15.5.2 Apparatus

(a) Air oven
(b) Homogenizer
(c) Pipettes 5, 10 and 25 mL
(d) Glass stoppered test tubes
(e) Volumetric flasks 10, 50, 100, 500 mL capacity
(f) Water bath
(g) Colorimeter

15.5.3 Reagents

(a) Zinc sulphate 23 % (w/v)
(b) Potassium Hexacyanoferrate 15.0 % (w/v)
(c) p- Dimethyl amino benzaldehyde in propanol 3 %(w/v)
(d) 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.
(e) Saturated Bromine water
(f) Ascorbic acid solution 10 %
(g) n- Butyl acetate
(h) Anhydrous Sodium Sulphate

15.5.4 Procedure

Weigh 5 gm of dried sample obtained after determination of moisture, transfer to the homogenizer, add 100 mL water and mix at the maximum speed for 1 minute. 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. Into a series of glass stoppered tubes, add by pipette 5 mL of test solution (containing 2 - 15 µg orotic acid), 5 mL of each of the standard orotic acid solutions and 5 mL of water to act as a blank. Add to each tube 1.5 mL of saturated bromine water and allow the mixture to stand at room temperature for not more than 5 minutes. 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 minute. 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. Cool to room temperature, add to each tube 4 mL n-butyl acetate and shake vigorously for 15 seconds. Transfer the upper separated layers to dry test tubes containing 1 gm anhydrous Sodium sulphate. Mix gently. Add another gram of anhydrous Sodium sulphate. Mix gently and allow to separate. Transfer the clear butyl acetate layer to 1 cm cell and measure the optical density at 461 - 462 nm against the blank.

15.5.5 Calculation

Draw a calibration graph of the standard orotic acid solution plotting the optical density on the X – axis against the concentration of orotic acid on the Y – axis. Determine the orotic acid content in 5 mL of sample extract by interpolation of the colorimetric reading on the calibration graph and hence the amount in the dry sample. For converting to milk assume that skim milk powder contains 62.5mg orotic acid per 100 gm.

(Ref: - IS 12711:1989 Bakery products – Methods of Analysis/Pearson Composition and Analysis of Foods 9th edn, page 316)

16. 0 CORN FLOUR, CORN FLAKES AND CUSTARD POWDER

16.1 Moisture

16.1.1 Procedure

Weigh accurately about 5 gm of sample in previously dried and tared aluminium or stainless steel dish (7.5 × 2. 5 cm with slip on cover) and place the dish with its lid underneath in an oven maintained at 105±1°C for 5 hours. Remove the dish, cool in a desiccator and weigh with the lid on. Repeat the process of heating, cooling and weighing at half hour intervals until the loss in mass between two successive weighings is less than 1 mg. Record the lowest weight.

16.1.2 Calculation

$$\text{Moisture \% by wt} = 100 \times \frac{(W1 - W2)}{W1 - W}$$

Where,

W1 = wt in gm of dish with sample before drying.

W2 = wt in gm of the dish with the sample after drying.

W = wt in gm of empty dish.

Note: - In case of Corn Flakes grind the sample to a fine particle size before taking sample for moisture test.

16. 2 Total Ash excluding Sodium Chloride

16. 2.1 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

16.2 2 Procedure

Ignite the dried sample left after determination of moisture in a muffle furnace at 550±60°C till a grey ash is obtained. Cool in a desiccators and weigh. Heat again in the muffle furnace for 30 minutes, cool and weigh again. Repeat this process until the difference between two successive weighs does not exceed 1 mg. Record the lowest weight. This is the total ash on dry wt. basis.

Dissolve the total ash in 25 mL of dilute Nitric acid (1+9). Filter through Whatman filter paper No 1 or equivalent collecting the filtrate in a 100 mL volumetric flask. Wash the contents thoroughly with hot water and make up the filtrate to 100 mL. To 25 mL aliquot of the filtrate add excess Silver nitrate solution (20 mL) stirring well to flocculate the precipitate. Filter and wash the precipitate with water. To the combined filtrate add 5 mL of concentrated Nitric acid and 5 mL of ferric alum indicator. Titrate the excess silver nitrate with standard ammonium thiocyanate till a light brown colour persists.

16 2.3 Calculation

Total ash on dry wt basis = $\frac{(W\,2 - W) \times 100}{W1 - W}$

Where W2 = Wt in gm of dish with the ash

W = Wt in gm of empty dish.

W1 = Wt in gm of dish with dried sample

Sodium Chloride on dry basis = $\frac{(V1\,N1 - V2\,N2) \times 5.85}{W1 - W} \times \frac{V3}{V4}$

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 = wt in gm of dish with sample

W = wt in gm 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 wt. basis.

16.3 Determination of Alcoholic acidity: Refer clause 15.3 above

17. 0 MALTED MILK FOOD

17. 1 Determination of Solubility

17.1.1 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.

17.1.2 Procedure

17.1.3 Reconstitution of Malted Milk Food

Weigh accurately 4 gm of the material into a 50 mL boiling tube, and add 32mL 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.

Fill the reconstituted milk into a 25 mL centrifuge tube and centrifuge for 10 minutes at 2000 rpm with a radius of 17 cm (giving a force of 770 gm). Cool in a refrigerator or in ice until the fat solidifies (taking care that milk does not freeze). Remove the fat layer with spoon shaped spatula. Bring the milk to room temperature (27±l°C). Break up the deposit with a glass rod. Cork the tube and shake vigorously until the liquid is homogenous.

17.1.4 Determination of Total Solids

Weigh 2 mL of homogenous liquid in a previously dried aluminium dish provided with a tight fitting lid (No. 1). Centrifuge the tube for 10 min. Without disturbing the sediment, pipette 2 mL of the supernatant into a second dish (No. 2) and weigh. Remove the lids of both the dishes (No. 1 and 2). Place on a water bath till the sample is dry. Keep the dishes in air oven at 98±2°C for 90 minutes, cool in a dessicator and weigh. Repeat heating and weighing till constant weight is obtained (within 2 mg).

17.1.5 Calculation

$$\text{Solubility \% by w/w} = \frac{W4 \times W1 \times 100}{W3 \times W2}$$

Where,

W1 = Weight of liquid taken in dish No. 1

W2 = Weight of liquid taken in dish No. 2

W3 = Weight of total solids in dish No. 1

W4 = Weight of total solids in dish No. 2

(Ref: - FAO Manuals of Food Quality Control 14/8 page 31/British standard 1743 : Part

2 : 1980)

17.2 Determination of Cocoa Powder

17.2.1 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

17.2.2 Reagents

(a) Concentrated Sulphuric acid - Approx 98 %

(b) Dilute alcohol – 80 % (v/v)

(c) Potassium Ferrocyanide -Dissolve 10.6 gm of crystallized Potassium Ferrocyanide in water and make upto 100 mL.

(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 – Dissolve 1 gm Methyl red in 200 mL of 95 % alcohol

(h) Zinc Acetate solution – Dissolve 21.9 gm of crystallized Zinc acetate and 3 mL glacial acetic acid in water and make upto 100 mL

(i) Magnesium oxide

(j) Standard Hydrochloric acid – 10 %

(k) Sucrose anhydrous , pure

(l) Selenium

17.2.3 Procedure

Grind 20 gm 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. Add 1 gm of freshly ignited Magnesium oxide and digest on a boiling water bath for 1½ hours using an air condenser and shaking occasionally. Filter while hot through a Buchner funnel, return the residue to the flask and digest again for 30 minutes with 50 mL of alcohol. Filter and repeat the digestion once more.

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, add 2- 3 mL of concentrated HCl and transfer the liquid to a 200 mL volumetric flask. Cool, add 5 mL of Zinc acetate, mix and add 5 mL of Potassium Ferrocyanide solution. Make up to mark and mix thoroughly. Allow the flask to stand for few minutes and filter through a dry filter paper.

Evaporate the whole of the filtrate to about 10 mL, transfer to a separatory funnel, and extract with with five successive 30mL portions of Chloroform, with vigorous and thorough shaking. 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. Dissolve the residue in a little hot water, transfer to a Kjeldahl flask, and add 0.2 gm sucrose and 10 mL of concentrated Sulphuric acid. Heat over a small flame until frothing ceases, add 0.2 gm selenium and digest until colourless.

Cool the contents of the flask. Transfer quantitatively to the round bottom flask with water, the total quantity of water used being 200 mL. 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. Assemble the apparatus taking care that the tip of the condenser extends below the surface of standard sulphuric acid contained in the flask. Mix the contents of the flask by shaking and distill until all the ammonia has passed over into standard sulphuric acid. Reduce the burner flame. Detach the flask from the condenser and shut off the burner. Rinse the condenser thoroughly with water into the flask. Wash the tip carefully so that all traces of condensate are transferred to the flask. 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.

17.2.4 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

$$\text{Cocoa powder \% by wt} = \frac{228.2\,(B - A)\,N}{W}$$

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 = weight in gms of the material taken for the test

18.0 DETERMINATION OF SYNTHETIC COLOUR IN BISCUITS, CAKES ETC

18.1 Apparatus

(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

18.2 Reagents

(a) 2 % ammonia in 70 % alcohol
(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) Chromatographic paper Whatman No 1 or equivalent

(d) 0.1 N Hydrochloric acid: 5 mL of concentrated HCl diluted to 1 L with water.

18.3 Procedure

Thoroughly grind 10 gm of powdered material with 50 mL of 2% ammonia in 70% alcohol. Allow to stand for few hours and centrifuge. Pour the clear supernatant liquid in the dish and evaporate on the water bath. Dissolve the residue in 30 mL water acidified with acetic acid.

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. 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. 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. Natural colour may also dye the wool during the first treatment but the colour is not removed by ammonia. 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. Pearsons chemical analysis of foods is recommended to be followed.

18.3.1 Separation of Colours by Paper Chromatography

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 from it. Pipette 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. Prepare 0.1% solutions of permitted dyes and with the help of a capillary tube, apply spots of all these dyes on the line leaving about 1.5 cm distance between two spots. Allow the coloured 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. The following solvent systems

may be used for separation of colours. Solvent 5 has been found to give good resolution.

(1) 1% ammonia = 1 mL ammonia (sp gr 0. 88) + 99 mL water

(2) 2.5% Sodium chloride

(3) 2% Sodium Chloride in 50% alcohol

(4) Isobutanol : Etahnol : Water (1: 2: 1 (v/v))

(5) n-Butanol : Water : Acetic acid (20 : 12: 5)

(6) Isobutanol :Ethanol : Acetic acid (3 : 12 : 5)

Close the chromatographic chamber tightly and let the solvent rise. When the solvent front has reached about 20 cm from the base line, remove the filter paper sheet and allow it to dry. 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. Allow the colour to extract and decant the coloured extract into a volumetric flask. Repeat the process of extraction and decanting till all the colour is removed from the paper. Make upto volume. Determine absorbance maxima and read the optical density at absorbance maximum against a blank prepared by cutting an equivalent strip plain portion of chromatogram and extracting it with 0.1 N HCl. From the absorbance values compute the concentration of the dye by reference to the plot of concentration versus optical density.

18.3.2 Plotting of standard curve

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. Read their absorbance at respective absorbance maxima. Plot absorbance values against concentration of the dye.

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

(Ref: - IS 12711: 1989 Bakery Products – Methods of Analysis)

19.0 SOLVENT EXTRACTED OIL SEED FLOURS - (SOYA FLOUR, GROUNDNUT FLOUR, SESAME FLOUR, COCONUT FLOUR, COTTONSEED FLOUR)

19.1 Determination of Total residual Hexane

19.1.2 Scope and field of application

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.

19.1.3 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.

19.1.4 Reagents and materials

(a) Technical 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) Auxillary gases

(1) Hydrogen 99.9 % pure, containing no organic impurities

(2) Air containing no organic impurities

19.1.5 Apparatus

i) Gas Chromatograph with flame ionization detector equipped with glass capillary column approx 30 metres long and 0.3 mm in diameter coated with methyl polysiloxane film of 0.2 µm thickness or failing this a packed column of at least 1.7 metre 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.

ii) Electric oven , capable of being maintained at 110°C

iii) Gas Syringe , graduated capacity 1 mL, preferably with a valve

iv) Penicillin type flasks of capacity 50- 60 mL all with the same volume to within 2 %.

v) Septa, inert to solvents, of approximately 3 mm thickness capable of producing a hermetic seal after crimping.

vi) Metallic foil caps of Aluminum

vii) Crimping pliers

viii) Liquid syringe 10 µm capacity.

19.1.6 Sampling and Sample storage

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.

19.1.7 Procedure

Weigh to the nearest 0.1 gm, 5 gm 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. 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. Set the oven temperature at 40°C, injector and detector temperature at 120°C, Carrier gas pressure at 0.3 bar (30 kPa).

Using the gas syringe previously heated to 50 – 60°C take exactly 0.5 mL of gaseous phase and inject quickly into the chromatograph.

19.1.8 Calibration

Three points with 2.5, 5.0, 10.0 µL of 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 gm.

Prepare a calibration series using flasks of the same capacity as used for the determination. Add to the flasks 6 mL of water followed immediately by various quantities of n–Hexane measured accurately with the help of the syringe. 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. At the end of this time remove the flasks from the oven and leave to cool for 2 minutes. With the gas syringe heated between 50 – 60°C take exactly 0.5 mL of headspace and inject quickly into the chromatograph. Carry out two determinations on the sample.

19.1.9 Expression of results

Construct the calibration graph by plotting the area of the solvent peak as a function of the mass of the solvent introduced into the flask (1 µL corresponding to 660

µg). Determine the sum of the peak areas of Hexane and various Hydrocarbons which usually make up the technical solvent. 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 is equal to $\frac{m_1}{m_0}$

Where,

m_0 = the mass in gm of the test portion.

m_1 = the mass in microgram of solvent present in the flask.

Take as the result the arithmetic mean of two determinations.

(Ref: - IS 12983: 1990/I.S.O 8892: 1987, Oilseed Residues – Determination of Total Residual Hexane)

20.0 DETERMINATION OF OXALIC ACID IN SOLVENT EXTRACTED SESAME FLOUR

20.1 Apparatus

(1) Waring Blender

20.2 Reagents

(1) Dilute Hydrochloric Acid (1+ 1)
(2) Ammonium hydroxide solution – sp gr 0.880
(3) Phosphoric Tungstate reagent – Dissolve 24 gm Sodium Tungstate in water. To this add 40 mL of syrupy phosphoric acid (sp gr 1.75) and dilute the solution to 1 L.
(4) Calcium Chloride Buffer solution – Dissolve 25 gm of anhydrous Calcium chloride in 500 mL of 50 % glacial acetic acid and add this solution to a solution of 530 gm of Sodium Acetate in water, diluted to 500 mL.
(5) Wash Solution – A 5% solution of acetic acid kept over calcium oxalate at room temperature. Shake the solution periodically and filter before use.
(6) Sulphuric acid -10% solution
(7) Potassium Permanganate solution – 0.02 N

(8) Capryl alcohol

20.3 Procedure

Homogenise about 6 gm of the sample with about 100 mL water in the blender and transfer the mixture to a 600 mL beaker with the minimum number of washings. Add 2 volumes of dil HCl to each 10 volumes of liquid (to give an approx normal concentration) and one or two drops of capryl alcohol and boil for 15 minutes. Allow to cool, transfer to a 500 mL volumetric flask, dilute to mark and after an occasional shaking set it aside overnight. Mix and filter through a dry filter paper. Transfer by means of a pipette 25 mL of filtrate into a tube fitted with a stopper, add 5 mL of phosphoric tungstate reagent, mix by inverting once or twice and set the mixture aside for 5 hours. Centrifuge for 10 minutes at 3000 rpm. 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 phosphotungstate. 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. Centrifuge for 10 minutes, carefully remove the washings, 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 0.02 N potassium permanganate.

20.4 Calculation

1 mL of 0.02 N Potassium Permanganate = 0.00090gm oxalic acid

(Ref: - IS specification No IS 6108 - 1971 Specification for Edible Sesame Flour (solvent extracted))

20.5 Estimation of oxalic acid in solvent extracted Sesame Flour by HPLC-UV Method

20.5.1 Apparatus

The analytical HPLC system equipped with a Waters 996 photodiode array detector. The separation was carried out using a Waters Atlantis dC18 steel cartridge (150 × 4.6 mm i.d.) with a particle diameter of 300 Å, using a Waters Atlantis (20 × 4.6 mm) dC18 guard column to protect the analytical column.

20.5.2 Reagents

1) Methanol of HPLC gradient grade,

2) Sodium dihydrogen phosphate (NaH_2PO_4) and phosphoric acid
3) Ethanol
4) Standards of cis-aconitic, fumaric, quinic, l-(+)-tartaric acids and
5) Sodium pyruvate
6) Ultrapure water

20.5.3 Procedure

Homogenize the samples (approximately 100 gm) till the formation of a homogeneous flour. Take five subsamples of fresh homogenised wheat to measure the pH and acidity. Determine the pH (2 gm of flour with 50 mL of ultrapure water) by potentiometric measurement at T =20^0C with pH meter (AOAC, 1990). Determine the acidity by means of titration with 0.1N Sodium hydroxide until pH 8.1. And the results are expressed in grams of anhydrous citric acid per kilogram (AOAC, 1990).

Determination of Organic acids by HPLC coupled to a diode array detector according to the method (Hernandez Suarez et al., 2008) previously optimised.

For each accession, weigh about 1 gm of homogenised flour directly in polypropylene tubes and mix with 2 mL of 80% ethanol. Afterwards, put the tubes into an ultrasound bath at 50°C for 5 min and then centrifuge for 5 minutes at 1090 × g. Carefully recover the supernatant to prevent the contamination with the dried pellet. Then, add another 2 mL of 80% ethanol to the pellet and place in an ultrasound bath and centrifuge as described above. Recover the two supernatants in the same tube. Concentrate this liquid phase with nitrogen stream until the elimination of all ethanol; adjust the residue to 5 mL with ultrapure water and store at T = -80° C in the freezer. Pass this dissolution through a 0.45-µm filter and through a strong anion-exchanger, which was previously preconditioned with 3 mL of ultrapure water. Elute the compounds by washing with two fractions of 1 mL of sodium dihydrogen phosphate (NaH_2PO_4 =20mm = to pH = 1). Perform duplicate injections and use average peak areas for the quantification.

- Mobile phase Sodium dihydrogen Phosphate (NaH_2PO_4 =20mm to pH = 2.7).
- The injection volumes 10µL of both the standard solutions and sample extracts,
- Flow rate: 0.7 mL min - 1.

- Wavelength: 210 nm

Identify the HPLC peaks by comparing the retention times and spectral data obtained from standards.

(Ref: International Journal of Food Science and Technology 2012, 47, 627–632)

Or

Nour, V. et al./Not. Bot. Hort. Agrobot. Cluj. HPLC Organic Acid Analysis in Different Citrus Juices under Reversed Phase Conditions.38 (1) 2010, 44-48.

Or

Vanda Pereira et al. HPLC-DAD methodology for the quantification of organic acids, furans and polyphenols by direct injection of wine samples. J. Sep. Sci. 2010, 33, 1204–1215.

21.0 DETERMINATION OF FREE AND TOTAL GOSSYPOL IN SOLVENT EXTRACTED COTTON SEED FLOUR

21.1 Free Gossypol

21.1 Definition

The term free gossypol defines gossypol and gossypol derivatives in cottonseed products which are soluble in aqueous acetone under the conditions of the method.

21.1.2 Apparatus

(1) Mechanical shaker to hold 250 mL Erlenmeyer flasks and provide vigorous shaking.
(2) Spectrophotometer isolating a band at 40 nm and equipped with cells of 1 cm light path or colorimeter equipped with a filter having maximum transmittance between 440- 460 nm.
(3) Grinding mill- with 1 mm screen
(4) Glass beads about 6 mm diameter.
(5) Erlenmeyer flasks 250 mL, pipettes, filter paper, medium retention , 11 cm size (Whatman No 2 or eqvt)
(6) Volumetric flasks 25, 200 ,250 mL, Class A
(7) Water bath for operation at 100°C equipped with clamps for supporting 25 mL

volumetric flasks.

21.1.3 Reagents

(1) Solvents

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) Aniline – distilled over a small amount of Zinc dust. Redistill when the reagent blank exceeds 0.022 absorbance (95 % transmittance)

(2) Thiourea solution – Dissolve 10 gm thiourea in water and make up to 100 mL

(3) 1.2 N HCl –Dilute 106 mL concentrated HCl (35-37%) to 1 L with water.

(4) Gossypol - Primary standard or gossypol acetic acid (89.61 % gossypol by wt) to be used for calibration.

(5) Standard Gossypol solution – Prepare by accurately weighing 25 mg primary standard gossypol or 27.9 mg gossypol acetic acid and transferring quantitatively to a 250 mL volumetric flask using 100 mL of acetone. Add 1 mL glacial acetic acid, 75 mL water, dilute to volume with acetone and mix well. 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. 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 hrs when protected from light.

21.1.4 Procedure

Grind about 50 gm sample in a Wiley grinding mill to pass 1 mm screen. 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 gm 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

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 hour. 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

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.

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. 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). Heat the sample aliquot B and the reagent blank in a boiling water bath for 30 minutes. 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

Determine the absorbance of sample aliquot A at 440 nm using aqueous isopropyl alcohol to set the instrument at zero absorbance (100% transmittance). 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. Determine the absorbance of sample aliquot B at 440 nm using the reagent blank to set instrument at 0 absorbance. Calculate the corrected absorbance of the aliquot as follows

Corrected absorbance = (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 a calibration graph prepared by taking1 , 2 , 3, 4, 5, 7 , 8, 10 mL aliquot of standard gossypol solution (0.025 mg/ml) into 25 mL volumetric flask. 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. 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. Prepare a reagent blank containing 10 ml of aqueous acetone, 2 drops of aqueous thiourea and 2 mL of aniline (do not add HCl). Heat the standards and the reagent blank in boiling water bath for 30 minutes, cool and dilute to volume with aqueous isopropyl alcohol and determine their absorbance . Determine corrected absorbance. Plot the corrected absorbance for each gossypol standard against mg of gossypol in 25 mL volume to obtain the calibration graph

(Ref: - AOCS (1989) Official Method Ba 8 – 78)

21.2Total Gossypol

21.2.1 Definition

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 diaminopropanel complex which then reacts with aniline to form dianilinogossypol under the conditions of the method.

21.2.2 Apparatus – same as in 21.1.2 above and pipettes 1, 2, 4, 5, 10 mL.

(1) Solvents – Isopropyl alcohol (n – propanol), n – hexane (b.p 68-69^{0}C), dimethyl formamide, 3 – amino 1 propanol (propanolamine), free of colour, glacial acetic acid and aniline. The aniline should be redistilled over zinc dust using water cooled condenser.

(2) Isopropyl alcohol- hexane mixture (60 + 40)

(3) 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.

(4) Gossypol or Gossypol acetic acid as primary standard.

(5) 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.

21.2.3 Procedure

Grind 50 gm sample in a Wiley mill to pass 1 mm sieve. Weigh 0.5 – 0.75 gm sample accurately and transfer to a 50 mL volumetric flask. Add 10 mL complexing reagent. Prepare reagent blank containing 10 mL of complexing reagent in a 50 mL volumetric flask. Heat sample and blank in a water bath at 100°C for 30 minutes, cool, dilute to volume with isopropyl alcohol- hexane mixture. Filter through 11 cm filter paper into a 50 mL glass stoppered Erlenmeyer flask discarding first 5 mL of the filtrate Pipette 2 mL of duplicate sample extract into 25 mL volumetric flasks. Pipette duplicate blank aliquots of same volume as sample aliquot into 25 mL volumetric flasks. Dilute one set of sample and blank aliquots with isopropyl – hexane mixture and reserve as

reference solutions for absorption measurement.

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. Allow to stand for 1 hour. Measure the absorbance at 440 nm of reagent blank treated with aniline using blank aliquot without aniline as reference solution. 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 21.1. 4 (free gossypol).

(Ref: - AOCS (1989) Official Method Ba 8 – 78).

21.3 Determination of Free and Total Gossypol in solvent extracted cotton Seed Flour by HPLC UV Method:

21.3.1 For free Gossypol

- HPLC - dual-wavelength absorbance detector,
- Zorbax Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm particle)
- C_{18} pre-column (4.6 mm × 20 mm, 5 μm) (Wang et al 1985).
- The mobile phase - 90 : 10 (v/v) methanol-0.5% acetic acid aqueous
- Solution flow rate of 0.8 mL/min.
- The wavelength for UV detection was 254 nm.
- Injection volume: 5 μL.
- The assays can be performed at room temperature.

(Ref: - Yingfan et al. J. Biosci. Vol. 29 No. 1, March, 67-71, 2004)

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