E-Practical Manual
on
Basic Food Analysis Laboratory Techniques (SFQ 003)
2(0+2)

B. Tech. (Food Technology)

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Preface

Food analysis is an important area to study the chemical and biochemical composition of food, required as a part of quality assessment of food. Assessment in Food Science and Technology requires determination of food composition and characteristic. As per the syllabus and the facility available in laboratories, practical experiments have been compiled in this manual which will be a useful resource to help undergraduate students.

Students belonging to **B.Tech. Food Technology** degree programmes taking it as Skill Enhancement course module will find it useful in learning various techniques by performing the experiments given in manual. It covers all the Practical's mentioned in the syllabus of **Basic Food Analysis Laboratory Techniques** along with some necessary information regarding the subject for the orientation of the UG students. The chapters focus on principles, procedure and applications of techniques as well as significance of experiments.

The authors believe that the students will get sufficient knowledge about basic food analysis methods applied in the field of Food Science & Technology. There may, however, be still some short coming in this book and the authors will be really grateful to receive suggestions from faculty, students, readers to make this manual better in future.

Ajay Kumar Maurya

List of Abbreviations

AOAC	Association of Official Analytical Chemists
FSSAI	Food safety and standard Authority of India
MC	Moisture content
NFE	Nitrogen Free Extract
COB	Clot on Boiling
TSS	Total soluble solids
N	Normality
M	Molarity
FDA	Food and drug Administration
pH	Potential of hydrogen
°B	Degree Brix
CFU	Colony Forming Unit
SPC	Standard Plate Count
BPW	Buffered Peptone Water
ICAR	Indian Council of Agricultural Research
Eq.	Equivalent
IS	Indian Standard

Basic Food Analysis Laboratory Techniques (SFQ 003)

2(0+2)

Syllabus

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Experiment 1

Objective: Determination of the moisture content in Food grains and Food grain products by Hot air oven method.

Principle

The sample is ground and a test portion is dried at a temperature of $130 \pm 3°C$ to a constant mass. The loss in weight is expressed as a percentage.

Requirements

1. Weighing balance
2. Petri dish
3. Hot air oven
4. Desiccator containing desiccant
5. Tong, Spatula

Procedure

1. Weigh 5 g of the food sample in the dish previously dried and weighed.
2. Place the dish in hot air oven at 130°C for 2 hours.
3. The time should be reckoned from the moment the oven attains 130°C after the dishes have been placed.
4. After 2 hours, transfer dish to desiccator and Cool in the desiccator.
5. When the dish has cooled to room temperature, weigh it & note the weight.
6. Place the dish again in oven, till a constant weight is achieved.

Calculation

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

Where,

W = Weight of the empty dish (g)

W1 = Weight of the dish with the sample before drying (g)

W2 = Weight of the dish with the sample after drying (g)

Observations

Sample No.	Weight of empty dish	Weight of the dish with the sample before drying (g)	Weight of the dish with the sample after drying (g)	Moisture Content
	W	**W1**	**W2**	**%**
A				
B				
C				

Result

The given food sample has ……………% moisture content.

Significance

      Moisture content in food has a significant impact on factors such as the product's taste, texture, appearance, shape, and weight. It has implications on legal and labelling requirements, the shelf life of the food or food products, food quality measurements, and food processing operations.

Hot Air Oven

Moisture content can be defined as the quantity of water present in a moist material.

Moisture content can be expressed in two ways:

- ➢ Wet basis
- ➢ Dry basis

Wet basis moisture content is the amount of water per unit mass of a moist substance.

$$MC_{wb} = \frac{MCdb}{1+ MCdb} \times 100$$

Dry basis moisture content is the amount of water per unit mass of dry solids.

$$MC_{db} = \frac{MCwb}{1- MCwb} \times 100$$

Mass of dry solid means moisture free as in oven dry or bone dry.

Moisture content dry basis is always greater than moisture content wet basis.

Wet and dry basis moisture content are interconvertible , but are not the same thing.

Moisture content dry basis can be greater than 100%

Generally wet basis moisture is used to describe the water content of agricultural materials and food products.

Q1. The moisture content of a sample was determined to 14% on wet basis. What is the moisture content on dry basis?

$$MC_{db} = \frac{MCwb}{1- MCwb} \times 100$$

Given,

MC_{wb} = 14% or 0.14

$$MC_{db} \ = \ \frac{0.14}{1- 0.14} \times 100$$

$$= \frac{0.14}{0.86} \times 100$$

$$= 16\%$$

Experiment 2

Objective: Determination of total Ash content in food grains and food grain products.

Principle

All organic matter is destroyed by ignition of the sample to a constant weight at high temperature of $550 \pm 25°C$ in a muffle furnace.

Requirements

1. Crucibles
2. Muffle furnace, capable of being regulated at 550 ± 25 °C
3. Desiccator, with desiccant
4. Weighing balance
5. Muffle furnace tongs for crucible, Spatula
6. Thermal protection gloves, capable to resist temperature up to 550-600 °C
7. Bunsen /Electrical burner

Procedure

1. Take 5gm dried sample in previously weighed empty crucible.
2. Ignite the dried material in the dish left after the determination of moisture with the flame of a burner till charred.
   Note: This step must be carried out in a fume hood
3. Transfer to a muffle furnace maintained at 550 ± 25 °C and continue ignition till grey ash is obtained.
4. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing at 30 min intervals till the difference in weight in two consecutive weighing is less than 1 mg.
5. Note the lowest weight.
   Note: 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 lightly grey.

Calculation

Total ash on dry basis (% by weight) $= \dfrac{W2 - W}{W1 - W} \times 100$

Where,

W = Weight in of empty Crucible (g)

W1 = Weight of the dish with the dried sample before ashing (g)

W2 = Weight of the dish with the dried sample after ashing (g)

Observation

Sample No.	Weight of empty Crucible	Weight of the dish with the dried sample before ashing (g)	Weight of the dish with the dried sample after ashing (g)	Ash Content
	W	W1	W2	%
A				
B				
C				

Result

The given food sample has ……………% Ash content.

Significance

Ash content in food represents the inorganic residue remaining after the organic matter has been burned off, which primarily consists of minerals.

Muffle Furnace

Experiment 3

Objective: Determination of crude protein in food sample by Kjeldahl method.

Principle

The sample is digested with concentrated H_2SO_4 using copper sulphate and potassium sulphate as a catalyst to convert organic nitrogen to ammonium sulphate. Alkali (NaOH) is added and it is converted into ammonia. The liberated ammonia is absorbed in excess of boric acid solution and then titrated against standard hydrochloric acid solution to determine the ammonia absorbed in boric acid.

Reagents required for digestion

Conc. Sulphuric acid

Catalyst Mixture 1:5 (Copper sulphate and Potassium Sulphate)

Reagents required for distillation

40% NaOH Solution

4% Boric Acid Solution

Mixed Indicator (Methyl red and Bromocresol green)

Reagents required for titration

0.1N HCL or 0.02N H_2SO_4

Apparatus

Kjeldahl tube, Digestion set, Kjeldahl Distillation unit, Burette, Pipette, Measuring Cylinder, Volumetric flask, Glass rod, Conical flask

Procedure

Digestion

1. Preheat the digestion set to 250°C
2. Weight 1gm of food sample and transfer to Kjeldahl tube
3. Add the sample with 2-3gm of catalyst mixture and 10ml conc. H_2SO_4 in the digestion tube and load the digestion in the digestion blocks
4. Ensure any frothing of sample are there, if the sample behaviour is normal then rise the temperature to 420°C.
5. Leave the tubes in blocks for 1hr, ensure the colour of sample turned to bluish green then remove the tubes and place in cooling stand.

Distillation

1. Load the digestion tube in distillation unit using the slider mechanism.
2. Keep empty 250ml conical flask and keep it in the receiver end.
3. Open the tap water for condensation.
4. Set the chemical requirement (Boric acid + Mixed indicator and NaOH) in to the microprocessor program according to the sample.
5. After distillation take the ammonia collection for titration and close the water tap.

Titration

The collected distillate solution will be in bluish green colour after titration with 0.1N HCL, it changes to pink.

Calculation

$$Nitrogen\% = \frac{14 \times Normality\ of\ Acid \times Actual\ Titrant\ Value \times 100}{Weight\ of\ sample \times 1000}$$

Protein % = Nitrogen % × Conversion Factor

Derivation factor

Usually, percentage nitrogen in protein is around 16. It means 1 gm nitrogen will be present in 100/16 = 6.25 gm of protein. Therefore, derivation factor is 6.25.

(14= Atomic weight of nitrogen)

Observation

Sample No.	Weight of sample (gm)	Volume made	Aliquot taken for distillation	Titre value	Nitrogen (%)	Crude protein (%)
A						
B						
C						

Result

The given food sample has ……………% crude protein.

Significance

It provides a measure of the total protein content in a sample, which is crucial for assessing the nutritional value of food and also plays a role in food labelling and quality control across various food products.

Kjeldahl unit

Experiment 4

Objective: Determination of crude fat in the given food sample by Soxhlet method.

Principle

The fat is extracted with petroleum ether from the oven dried sample. The solvent is removed by evaporation and residue of fat is weighed.

Apparatus

Soxhlet, Hot air oven, Thimbles, Weighing balance

Reagents

40 - 60°C anhydrous petroleum ether

Procedure

1. Weight 5gm of dried sample in an extraction thimble.
2. Plug the thimble with cotton and place it in the extractor.
3. Take the initial weight of flask.
4. Drop the thimble into the fat extraction tube to a Soxhlet flask
5. Pour 75ml anhydrous petroleum ether through the thimble into the flask.
6. Ensure proper sealing of flask.
7. Attach the top of the fat extraction tube to the condenser.
8. Extract the sample under reflux for 5-6 hours under boiling condition.
9. At the end of extraction period, increase the temperature to recovery temperature to evaporate the solvent.
10. When the level of solvent touches below the thimble, open the stopper slowly and allow the recovered solvent to flow drop by drop through the thimble.
11. Ensure complete recovery of solvent in the condensation compartment.
12. Take out beaker from the system.
13. Take out thimble from the beaker.
14. Keep the beaker inside the hot air oven at 100°C for few minutes to remove solvent vapours.
15. Take the final weight of beaker.
16. Place the empty beaker into the system.
17. Open the stopper and flow the solvent into the beaker.
18. The recovered solvent can be used for further extractions.

Calculation

Crude fat (%) = $\dfrac{W2 - W1}{W} \times 100$

Where,

W = Weight of sample (g)

W1 = Weight of empty flask (g)

W2 = Weight of flask with fat (g)

Observation

Sample No.	Weight of Sample	Weight of empty flask (g)	Weight of flask with fat (g)	Crude fat
	W	**W1**	**W2**	**%**
A				
B				
C				

Result

The given sample of food has ……………% crude fat.

Significance

　　　It provides a measure of the total fat content within a sample, which is crucial for various applications including food labelling, quality control in food production and research as it allows for assessment of nutritional value, compliance with regulatory standards, and understanding of the impact of fat content on product characteristics and consumer health.

Soxhlet

Experiment 5

Objective: Determination of crude fibre in the given food sample by FibroTRON apparatus.

Principle

Crude fibre is the organic matter remains after ignition of dried residue remaining after digestion of sample with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH solutions under specific conditions. Separation of the residue by filtration followed by drying and ashing of the residue. The loss in weight resulting from ashing corresponds to the crude fibre content of the sample.

Apparatus

1. FibroTron apparatus
2. Crucibles
3. Desiccator
4. Weighing Balance
5. Hot air oven
6. Muffle furnace

Reagents

1. H_2SO_4 (Sulfuric acid) 1.25%
2. NaOH (Sodium hydroxide) 1.25%
3. Distill water

Preparation of Reagents

Sulphuric acid (H_2SO_4) solution, 0.255 N: Add about 200 mL of distill water into 1000 mL volumetric flask and add 12.5 g of conc. Sulfuric acid and make up the volume with distill water

Sodium hydroxide (NaOH) solution, 0.312 N: Add about 200 mL of distill water into 1000 mL volumetric flask and add 12.5 g of Sodium hydroxide pellets and make up the volume with distill water.

Procedure

1. Weigh accurately about 2g fat free sample and transfer to crucible.
2. Place the crucible into rubber adaptor of FibroTRON extraction unit.
3. Pour 100 ml of 1.25% H_2SO_4 into the extractors from the top of the system.
4. Boil at 350°C then reduce the temperature to 250°C and boil for 30 min.

5. After 30 min boiling, drain the acid and wash the sample twice or thrice with distill hot water.

6. During draining ensure that the knob is in vacuum mode.

7. Pour 100 ml of 1.25% NaOH (sodium hydroxide) into the extractors from top of the system.

8. Boil at 350°C then reduce the temperature to 250°C and boil for 30 min.

9. After 30 min boiling, drain the alkali and wash the sample twice or thrice with distill hot water.

10. During draining ensure that the knob is in vacuum mode.

11. After alkali wash take out the crucibles and dry them in hot air oven until the crucibles are free from moisture.

12. Cool the hot crucible to room temperature using a desiccator.

13. Take the weight of crucible.

14. Place all the crucibles in the muffle furnace at 450°C for ashing until all carbonaceous matter is burnt.

15. Cool the crucible into desiccator.

16. Take weight of crucible.

Calculation

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

Where,

W = Weight of sample (g)

W1= Weight of crucible before ashing (g)

W2= weight of crucible after ashing (g)

Observation

Sample No.	Weight of Sample (g)	Weight of crucible before ashing (g)	Weight of crucible after ashing (g)	Crude fibre
	W	W1	W2	(%)
A				
B				
C				

Result

The given food sample has ……………% crude fibre.

Significance

It provides a measure of the indigestible plant cell wall components (mainly cellulose, hemicellulose, and lignin) in a food which is crucial for assessing its nutritional value, food quality as it indicates the portion of the food that cannot be readily digested and utilized for energy, while still playing a vital role in gut health and digestion regulation by stimulating gut motility.

FibroTRON

Experiment 6

Objective: Calculation of carbohydrate (%) in the food sample by difference method.

Total carbohydrate content of foods has been calculated by difference, rather than analysed directly. Under this approach, the other constituents in the food (protein, fat, moisture and ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula:

Calculation

Total Carbohydrate (%) = 100 – (Moisture % + Ash % + Fat % + Protein %)

Nitrogen free extract (NFE) or soluble carbohydrates

Available carbohydrate represents that fraction of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism. (It does not include dietary fibre, which can be a source of energy only after fermentation). To calculate available carbohydrate by difference, the amount of dietary fibre is analysed and subtracted from total carbohydrate, thus:

NFE% = 100 – (Moisture % + Ash % + Fat % + Protein % + Crude fibre)

Observation

Sample No.	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fibre (%)	Carbohydrate (%)	NFE (%)
A							
B							
C							

Result

The given food sample has ……………% total carbohydrates.

Significance

It allows for the accurate determination of the amount of carbohydrates in a food sample, which is crucial for nutritional labelling, quality control, understanding food composition, and managing dietary needs, particularly for individuals with conditions like diabetes.

Experiment 7

Objective: Determination of Gluten content in wheat flour.

Principle

This test involves forming a dough and washing out the starch and water-soluble components (e.g. water-extractable pentosans, sugars, water-soluble proteins) from the dough. A wet and elastic substance made of water-insoluble proteins (gliadins and glutenins, about 85% total protein) remains after the washing procedure whose amount is an indication of gluten quantity.

Apparatus

1. Weighing balance
2. Spatula
3. Desiccator
4. Petri dishes
5. Oven
6. Tongs
7. Silk cloth

Reagents

1. Potassium iodide
2. Iodine

Preparation of reagents

2 % iodine in Potassium iodide solution: Dissolve 10 grams of potassium iodide crystals in 100 ml distilled water. Swirl until crystals dissolve. Add 2 grams iodine and swirl until all iodine dissolves. Store in a dark brown bottle in the dark.

Procedure

1. Weigh 25g wheat flour sample.
2. Add tap water to form a dough.
3. Knead the dough by hand until a firm to medium consistency dough ball is obtained.
4. Keep the dough gently in a beaker filled with water and let it stand for 60 min.
5. Remove the dough and place it in a piece of silk cloth.
6. Wash under gentle stream of tap water until all starch is washed out and wash water is clear.

7. Water passing through the silk cloth does not turn blue when a drop of iodine solution is added in it.

8. When much of the starch has been removed, the gluten ball will become darker and more elastic.

9. Collect the residue to form a ball, squeeze in the palms to remove excess water.

10. Transfer gluten ball to a previously dried and weighed petri dish and keep it in the oven at 130°C for drying.

11. When partially dried, remove and cut into several pieces with a scissor and again keep in the oven to dry.

12. Cool in a desiccator and weigh.

13. Return it to the oven again for 30 min, cool and weigh to ensure constant weight.

Calculation

Gluten (%) on wet basis $= \dfrac{W1}{W} \times 100,$

Gluten (%) on dry basis $= \dfrac{W2}{W} \times 100$

Where,

W = Weight of sample (g)

W1= Weight of gluten before drying

W2= Weight of gluten after drying

Observation

Sample No.	Weight of Sample (g)	Weight of gluten before drying (g)	Weight of gluten after drying (g)	Gluten on wet basis	Gluten on dry basis
	W	**W1**	**W2**	**%**	**%**
A					
B					
C					

Result

The given food sample has ……………% gluten content on dry basis and …………% on wet basis.

Significance

It affects the texture, flavor, and nutritional value of many foods. It also determines whether a food can be labelled as "gluten-free". The FDA and FSSAI have set a regulatory limit of 20 mg/kg of gluten for foods to be labelled as "gluten-free". Gluten can be a problem for people with celiac disease, non-celiac gluten sensitivity, or wheat allergies.

Experiment 8

Objective: Determination of pH of the given food sample.

pH is defined as the logarithm of reciprocal of hydrogen ion concentration in gm/ litre. It can be measured directly using pH meter.

The basic principle of potentiometry involves the use of an electrolytic cell composed of two electrodes dipped into a test solution. A voltage develops, which is related to the ionic concentration of the solution. Since the presence of current could alter the concentration of surrounding ions or produce irreversible reactions, this voltage is measured.

Reagents

Standard Buffer solution (pH 4, 6)

pH meter, conical flask, beaker

Procedure

1. Switch "ON" the mains then press the switch "ON/OFF" of the pH meter.
2. Take 10 ml sample in a beaker.
3. Rinse the pH electrode with distilled water. Place the electrode and temperature probe into the sample solution and stir gently.
4. The pH sensitive glass membrane and liquid junction must be completely immersed into the solution.
5. If the Auto- Hold option in the setup menu is enabled, the meter will automatically sense a stable reading and lock measurement, the ready icon appears on the display.
6. Press the Meas key to resume measuring. Wait for the measurement to stabilize and record the reading.
7. Rinse the pH glass electrode and temperature probe 2-3 times with distill water and wipe dry with tissue paper.
8. Dip the glass electrode in 0.3 M KCL when not in use.

Observation

Sample No.	pH
A	
B	
C	

Result

The given food sample has ……………

Significance

It measures the acidity or alkalinity of a solution, which plays a crucial role in various biological, chemical, and environmental processes, impacting factors like nutrient availability, microbial activity, chemical reactions, and even the quality and safety of food and food products.

pH Meter

Experiment 9

Objective: Determination of titratable acidity of the given food sample.

Principle

The acid present in the sample determined by titration against standard base and stated in terms of predominant acid in the sample. Titratable acidity (also called total acidity) measures the total acid concentration in a food.

Reagents

1. Sodium hydroxide 0.1N
2. Phenolphthalein solution 1%

Apparatus

Volumetric flask 25ml, Burette, Pipette, 100ml Conical flask

Procedure

1. Weigh 5gm sample and add distilled water.
2. Transfer to a 25ml volumetric flask and make up to the volume.
3. Filter through Whatman No. 1 filter paper.
4. Take 10ml aliquot in a conical flask and add few drops of phenolphthalein.
5. Titrate it with 0.1N NaOH solution to give pink colour.
6. Note the Volume of 0.1N NaOH used in titration.

Calculation

$$\text{Total acid (\%)} = \frac{Titre\ value \times Volume\ made\ up \times Normality\ of\ alkali \times Eq.wt.of\ acid \times 100}{Aliquot\ taken\ for\ titration \times Wt.of\ sample \times 1000}$$

Observation

Sample No.	Weight of sample taken	Volume made up	Aliquot taken for estimation	Titre Value	Titratable acidity (%)
A					
B					
C					

Result

The given food sample has ……………% titratable acidity.

Significance

It helps in determining taste, consistency, and appearance, and it can also helpful in monitoring foods quality that change acidity over time. Determines freshness of certain foods like milk. Titratable acidity is a better predictor of acid's impact on flavor than pH.

Experiment 10

Objective: Determination of total soluble solids (TSS) in fruit juices.

Principle

A digital refractometer measures the refractive index of a sample by passing light through a prism and detecting the reflected light. The refractive index is a measure of how fast light passes through a substance. TSS also includes small amounts of other soluble components like organic acids, proteins, and minerals.

Apparatus

Abbe or Hand Refractometer, Digital refractometer

Procedure

1. Inspect the prism window to ensure it is clean and dry.
2. Using a plastic pipette, drip sample onto the prism surface. Fill the well completely.
3. Press the Read button; the instrument begins to measure and the results is shown in the display.
4. Remove sample from the sample well by absorbing with a soft tissue.
5. Using a plastic pipette, rinse prism and sample well with distilled or deionised water. Wipe dry. The instrument is ready for the next sample.

Note: Degrees Brix (°B) is a measure of the dissolved solids in a liquid, and is commonly used to measure dissolved sugar content of a solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass.

Soluble solids may be determined by means of brix hydrometer which measure specific gravity or refractometer which measures refractive index. Brix hydrometer gives directly the % of sugar by weight in the syrup. Abbe refractometer gives refractive index as well as brix.

Observation

Sample No.	TSS
A	
B	
C	

Result

The given food sample has ……………°B TSS.

Significance

It indicates the concentration of sugars and other soluble components within a product, essentially reflecting its sweetness level and maturity stage, thus playing a crucial role in determining optimal harvest time and overall taste quality.

Digital Refractometer

Experiment 11

Objective: Measurement of physical properties of food grains.

Principle

Bulk density, true density and porosity form an important parameter for the design of storage requirements for food grains and also an indicator of its quality for purposes of grading. Material with low bulk density will require large space for the same weight as compared to material with high bulk density. Similarly true density and porosity also indicate the soundness of grains.

Requirements

Measuring cylinder 100 ml capacity, Weighing balance and Toluene solution

Bulk Density

It is density of grain including air space. Take a measuring cylinder of 100 ml capacity and fill it with grains for which density is to be measured. The measuring cylinder should be filled to its highest mark. Adjust the level of grains by repeated tapping. Take the weight of these grains in weighing balance. Repeat the reading five times.

$$\text{Bulk Density(g/cm}^3) = \frac{M}{V}$$

Where,

M = Weight (g) of the sample

V = Volume of the sample

True/ Particle density

True density or solid density, was determined by toluene displacement method. Fill the measuring cylinder with toluene up to certain level. Weight 25 g of sample, pour it in measuring cylinder containing toluene and note the volume of toluene.

$$\text{True density (g/cm}^3) = \frac{M}{V2 - V1}$$

Where,

M = Weight of sample,

V1 = Initial volume of sample

V2 = Final volume of sample

Porosity

Porosity (%) indicates the number of pores in the bulk material and calculated as

$$\text{Porosity} = \left\{ 1 - \frac{\text{bulk density}}{\text{true density}} \right\} \times 100$$

1000 Kernel Weight

Select 100 count grains & find their weight and multiply by 10.

Observation

Sample No.	Weight of sample	Volume of sample	Bulk Density	True density	Porosity
	g)		g/cm³	g/cm³	(%)
A					
B					
C					

Result

The given food sample has ……………

Significance

The physical properties of grains are important for designing equipment to handle, store, and process them. Physical properties also help estimate grain quality and determine how to transport and store them. The grains' size, density, and porosity are essential parameters to know during equipment design and agricultural processes like sorting, mixing, storing, and transporting grains.

Bulk density

A measure of how tightly packed the grains are, which can be affected by moisture, insect infestation, and foreign matter

Porosity

Affects the rate of heat and mass transfer of moisture during drying and aeration

Experiment 12

Objective: To perform clot on boiling (COB) test of given milk sample.

Principle

The COB test is based on observation that milk sample having high developed acidity due to bacterial growth will coagulate at high temperature.

Requirement

Test tube, Pipette, Water bath

Procedure

1. Transfer 5 ml of milk sample into a test tube and place it in a water bath at 100°C for 5-6 min.
2. Remove the test tube from water bath & rotate it in an inclined position.
3. Examine the films on the sides of the test tube for any precipitated particles.
4. Formation of clots or appearance of precipitation indicates that the COB test is positive.

Observation

Sample No.	COB Positive	COB Negative
A		
B		
C		

Significance

It is used to assess the quality of milk by checking its heat stability, essentially determining if the milk will curdle or form clots when heated. Milk with COB positive test indicates developed acidity (as lactic acid) above 0.17% and is unsuitable for processing and distribution.

Water bath

Experiment 13

Objective: Identification of Gram$^+$ and Gram$^-$ bacteria by staining method.

Principle

Gram-positive bacteria have more peptidoglycan in their cell walls, while Gram-negative bacteria have more lipids. The decolourizer shrinks the peptidoglycan layer, making it harder for the crystal violet-iodine complex to penetrate.

Reagents: Primary dye

Solution A: Dissolve 2gm crystal violet in 20ml of ethyl alcohol

Solution B: Dissolve 0.8gm of ammonium oxalate in 80 ml of distill water. Mix equal quantity Sol. A & Sol. B to prepare primary dye.

Mordant

Dissolve 1gm iodine & 2gm of potassium iodine in 300ml of distill water.

Counter stain: 10 ml of 2.5% solution of safranin in 95% ethyl alcohol with 100ml of distill water.

Procedure

The Gram staining process includes four basic steps, including

1. Applying a primary stain (Crystal violet)
2. Adding a mordant (Gram's iodine)
3. Rapid decolorization with ethanol, acetone or a mixture of both
4. Counter staining with safranin

Staining:

1. Clean the glass slide and dry it.
2. Flame the surface of slide and transfer the bacterial colony on to the surface of slide by means of sterile loop and spread on slide (smear) until dry.
3. Put primary dye solution on the slide and let it stand for 1 minute.
4. Rinse the slide with water to remove excess dye.
5. Put Mordant solution on the slide and let it stand for 1 minute.
6. Rinse the slide with water to remove excess iodine.
7. Decolorize by adding alcohol drop by drop on the slide until free colour is removed.
8. Rinse the slide immediately with water.
9. Add Counterstain to the slide for 1-2 minutes and wash with water.

10. Allow it to dry for 2-3hrs.

Microscopic Examination

1. Examine the slide under the microscope.

2. Cells which do not decolorise & retain the blue colour are Gram$^+$ bacteria.

3. Cells which decolorise & accept safranin are gram$^-$ bacteria.

Observation

Sample No.	Gram Positive	Gram Negative
A		
B		
C		

Significance

It helps identify bacteria in food samples, which can help determine if there is a bacterial contamination or infection.

Experiment 14

Objective: Enumeration of total viable count by standard plate count method in the given food sample.

Principle

A specified quantity of homogenised food sample is either dispensed into an empty Petri dish, mixed with a specified molten agar medium or surface plated on a solid agar culture medium. Other plates are prepared under the same conditions using decimal dilutions of the test sample or of the initial suspension. The plates are incubated under aerobic conditions at 30 - 37°C for 24 hr. The number of microorganisms per gram or millilitre of sample is calculated from the number of colonies obtained on the plates.

Reagents

Nutrient agar media

Buffered Peptone Water (BPF)

Apparatus

1. Laminar airflow

2. Hot air oven

3. Autoclave

4. pH meter

5. Incubator (30 -37°C)

6. Water bath

7. Refrigerator

8. Petri dishes

9. Micropipette with tips

10. Tubes and glass bottles

11. Vortex

12. Mechanical stirrer

13. Colony counter

Procedure

Sample preparation

1. Weigh the 25gm of food sample aseptically into a sterile blender jar & dissolve it in 225 ml (BPW) buffered peptone water.

2. Blend the food at a speed of 15000rpm- 2000rpm for not more than 2-5min

Media preparation

1. Ensure the initial culture is well-mixed to represent the diversity of microorganisms present.

2. Remove the stopper & flame the mouth prior to use, place the required number of nutrient agar tubes in boiling water until the medium completely melts & then cool to 45 – 50 °C.

3. Allow the medium to remain in the liquid state at this temperature.

Serial Dilution:

1. Prepare a series of decimal dilutions of the sample by transferring 1ml of the well shaken sample and adding it to 9ml of BPW & so on, using a fresh sterilized pipette for each dilution & ensuring through mixing to achieve a uniform distribution of microorganisms.

2. Using separate sterile pipettes, prepare decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and others as appropriate, of food homogenate.

3. Prepare all decimal dilutions with 9 ml of sterile diluent plus 1 ml of previous dilution, unless otherwise specified, by transferring 1 ml of previous dilution to 9 ml of diluent.

Inoculation and incubation

Pour Plate Technique

1. Inoculate at least two petri dishes with 1ml each of the different dilutions.

2. Transfer 1 ml of test sample if liquid, or 1 ml of the initial suspension (10^{-1} dilution) in the case of other products to sterile petri dishes in duplicate.

3. If more than one dilution is to be plated, the number of dishes can be reduced to one.

4. Similarly transfer 1ml of suspension from subsequent dilutions (10^{-2}, 10^{-3} or higher) to separate sterile petri dishes using a new sterile pipette for each decimal dilution.
   Note: If appropriate and possible, select only the critical dilutions steps (at least two consecutive decimal dilutions) for the inoculation of the Petri dishes that will give colony counts of between 10 and 300 colonies per plate.

5. Add not less than 10 ml to 15 ml of the melted nutrient agar at 44°C to 47 °C into each petri dish within 15 min of original dilution.

6. The time elapsed between sample preparation and plating shall not exceed 45 min.

7. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface and allow the mixture to solidify by leaving the petri dishes standing on a cool horizontal surface.

8. Invert solidified petri dishes, and incubate promptly for 24 ± 2 hr at 37°C.

9. Remove the plate from the incubator after 24 ± 2 hr incubation.

Counting the colonies

1. Count the colonies with visually or with a colony counter.

2. The best plates to count are those with 30-300 colonies.

3. Count all the colonies in these plates with less than 30 colonies. Count each spreader as one colony.

4. Plates with spreading colonies may be avoided if the colony occupies more than 1/4th of plate area.

Calculation

Colony forming unit (/gm or ml) = Colonies counted × reciprocal of dilution factor

Dilution factor = ml plated × dilution plated

Observation

Dilution	No. of colony counted	Total colony counted (CFU/ml)

Significance

It provides a basic measure of the total viable bacterial population within a sample, offering insights into the overall microbial quality and potential contamination risks in food products.

Autoclave   **Laminar flow**

Experiment 15

Objective: Determination of sensory quality to describe the flavour profile of given sample.

Sensory Evaluation

Sensory evaluation is a scientific discipline that analyses & measures human responses to the composition of food & drink. e.g. Appearance, Touch, Odour, Texture, Taste.

Procedure

Smell the given sample & identify the aroma perceived. The aroma can be identified in identical forms or as a flavour describing any other similar compound.

Observation

S. No.	Sample code	Flavour identified	Results
1	ABA		
2	ABB		
3	ABC		
4	BCA		
5	BCB		
6	CBA		
7	CAB		

Significance

It allows companies to understand how consumers perceive a product using their senses (sight, smell, taste, touch, and hearing), providing crucial information for product development, quality control, and market research by identifying consumer preferences and guiding improvements to ensure a product is well-received by the target market.

Experiment 16

Objective: Detection of adulteration in the given food sample.

Adulteration

Food adulteration is the intentional addition of inferior substances or removal of vital element from food to change its quality or nutritional value. This is done to increase profits or to make food look more appealing.

1. Adulteration of starch in milk

Principle

Starch in milk is detected by iodine test, where blue colour develops on addition of iodine in starch solution. The development of blue colour is due to complex formation between iodine and amylose component of starch. Heating the solution containing starch-iodine complex also destroys the colour although reversibly.

**Apparatus/reagents-** Test tube, Pipette, Volumetric flask, Water bath

Iodine solution: 1 gm iodine & 5 gm of potassium iodide in distill water and dilute to 100ml

Procedure

1. Take 3ml milk sample in a test tube.
2. Boil the content over a flame or water bath.
3. Cool to room temperature.
4. Add 1 drop of iodine solution.
5. Observe the colour.

Observation

1. Appearance of blue colour indicates the presence of starch in milk
2. Blue colour disappear on heating & reappears on cooling.
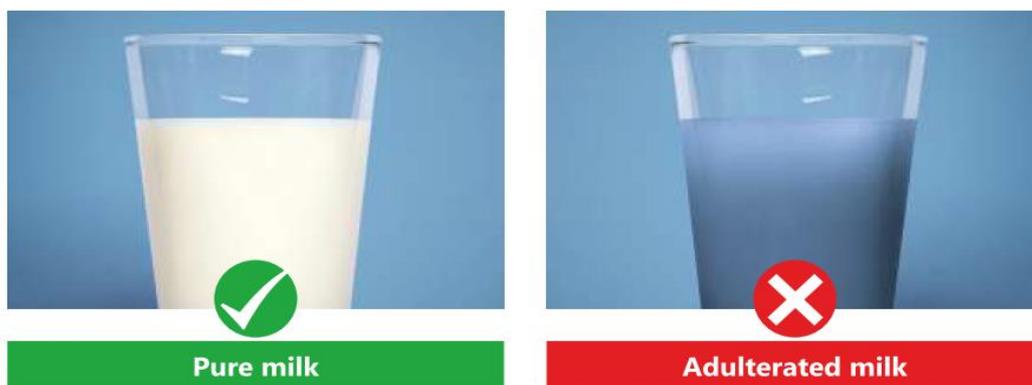
Source: DART, FSSAI

Significance

Starch or flour is added to increase the density/ specific gravity of milk to prevent detection of added water.

2. Adulteration of detergent in milk.

Principle

Detergent gives pink colour in presence of phenolphthalein solution. Detergents may be intentionally added in milk to emulsify the added vegetable oil in milk. Detergents are also believed to be part of the so called "synthetic milk" formulation.

Apparatus/reagents- Test tube, Pipette, Volumetric flask
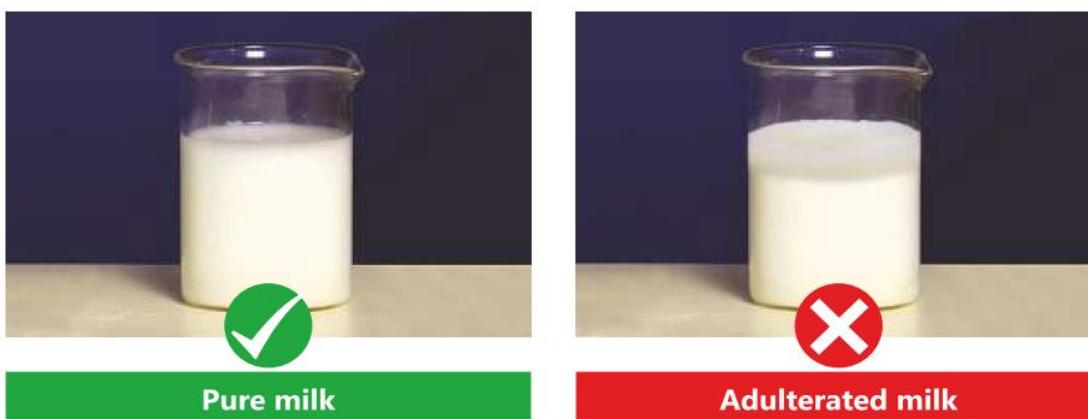
Phenolphthalein solution

Procedure

1. Take 5ml of milk in a test tube.
2. Add equal amount of hot distill water.
3. Add 1-2 drops of phenolphthalein solution.

Observation

Appearance of pink colour indicates the colour of detergent in milk.

Source: DART, FSSAI

Significance

Detergents are added to milk to emulsify oil and water, which makes milk frothy and white. It protects public health, consumer trust and economic losses.

List of Acids and Alkali with their equivalent weight.

S. No.	Acids/Alkali	Equivalent weight (g)
1	Hydrochloric acid (HCl)	36.47
2	Sulfuric acid (H_2SO_4)	49.04
3	Phosphoric H_3PO_4	32.67
4	Citric (anhydrous) ($H_3C_6H_5O_7$)	64.04
5	Citric (hydrous) ($H_3C_6H_5O_7$)	70.05
6	Acetic ($HC_2H_3O_2$)	60.05
7	Lactic ($HC_3H_5O_3$)	90.08
8	Malic ($H_2C_4H_4O_5$)	67.05
9	Oxalic ($H_2C_2O_4$)	45.02
10	Tartaric ($H_2C_4H_4O_6$)	75.05
11	Ascorbic ($H_2C_6H_6O_6$)	88.06
12	Sodium hydroxide (NaOH)	40
13	Sodium carbonate (Na_2CO_3)	53

Source: Adapted and modified from Nielsen (2010)

Annexure II

List of food products with their conversion factor for crude protein estimation.

S. No.	Food products	Conversion factor
1	Eggs	6.25
2	Meat	6.25
3	Milk	6.38
4	Barley	5.83
5	Corn (maize)	6.25
6	Millets	5.83
7	Oats	5.83
8	Rice	5.95
9	Rye	5.83
10	Sorghums	6.25
11	Wheat: Whole kernel	5.83
12	Wheat Bran	6.31
13	Endosperm	5.70

Source: Adapted from Merrill and Watt (1973)

Annexure III

Acid composition of important fruits

S.No.	Name of Fruits	Principal acid	Acid (%)
1	Apples	Malic	0.27–1.02
2	Bananas	Malic/citric (3:1)	0.25
3	Cherries	Malic	0.47–1.86
4	Cranberries	Citric	0.9–1.36
		Malic	0.70–0.98
5	Grapefruit	Citric	0.64–2.10
6	Grapes	Tartaric/malic (3:2)	0.84–1.16
7	Lemons	Citric	4.2–8.33
8	Limes	Citric	4.9–8.3
9	Oranges	Citric	0.68–1.20
10	Peaches	Citric	1–2
11	Pears	Malic/citric	0.34–0.45
12	Pineapples	Citric	0.78–0.84
13	Raspberries	Citric	1.57–2.23
14	Strawberries	Citric	0.95–1.18
15	Tomatoes	Citric	0.2–0.6

Source: Adapted from Nielsen (2010)

Annexure IV

Preparation of sodium hydroxide solution of approximate normality

Normality

It is also defined as "number of gram equivalents of solute per litre of the solution". 1N solution contains 1 gram-equivalent weight of solute per liter of solution.

It can be calculated using the formula:

Normality = grams of solute x 1000 / equivalent weight x volume of solution   OR

Weight of Acid/Alkali (g) $= \frac{NEV}{1000}$

Where,

N= Normality

E= equivalent weight

V= Volume made

The normality formula states that the normality of one solution multiplied by its volume equals the normality of a second solution multiplied by its volume. Acid strength is frequently reported in normality (equivalents per liter) and can be calculated

$N_{titrant} \times V_{titrant} = N_{sample} \times V_{sample}$

Where N is normality and V is volume (often in milliliters).

Preparation of 1 N solution of NaOH

For 1 Normal solution we need to know the, equivalent weight of NaOH, which is calculated by dividing Molecular weight by 1, that is 40 divided by 1= 40g

To make 1 N solution, dissolve 40g of sodium hydroxide in water to make volume 1 liter.

Standardization of prepared sodium hydroxide solution of approximate normality

Standardization simply is a way of checking our work, and determining the exact concentration of our NaOH (or other) reagent. Maybe our dilution was inaccurate, or maybe the balance was not calibrated and as a result the normality of our sodium hydroxide solution is not exactly 1 N as we intended. So, we need to check it. This is achieved by titrating the NaOH solution with an acid of known strength (Normality).

1. Fill the burette with prepared standard sodium hydroxide solution up to a known mark.
2. Transfer accurately weighed appropriate quantity of potassium acid phthalate (Molecular weight: 204.229) in 300 ml flaks and add 50 ml of distilled water.
3. Swirl the flask gently until potassium acid phthalate dissolves.
4. To it add 2-3 drops of phenolphthalein indicator.
5. Note down the initial reading on the scale of burette and then titrate with sodium hydroxide solution until the colour changes from colourless to pink.
6. Note down the final reading on the burette.
7. Determine the actual normality of the prepared sodium hydroxide solution using following equation:

$$\frac{\text{Weight of potassium acid phthalate (g) X 1000}}{\text{Volume of sodium hydroxide used X (204.229)}}$$

Note:

If the Normality of the sodium hydroxide calculated is not same as intended, then dilute it accordingly to have required normality using the following equation

$N_1 V_1 = N_2 V_2$

Where,

N_1 = required normality of the solution

V_1 = required volume of solution

N_2 = Normality of prepared solution

V_2 = Volume of prepared solution to be dilute

To check the normality of the final sodium hydroxide solution, again repeat the standardization as indicated above.

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