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Food Safety and Standards Authority of India
(A Statutory Authority established under the Food Safety & Standards Act, 2006)
Quality Assurance Division
FDA Bhavan, Kotla Road, New Delhi-110 002

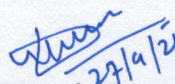
Dated, the 27th September, 2021

Notice

Inviting "Public Comments on Revised Manual of Methods of Analysis of foods – Dairy and Dairy Products"

A Revised Manual of Methods of Analysis of foods – Dairy and Dairy Products has been approved by the Scientific Panel on Methods of Sampling & Analysis in its 34th meeting held on 27.07.2021

2. Before the same are finalized, the comments/ views/ suggestions of the stakeholders are solicited.
3. The comments/suggestions; if any, should be furnished to FSSAI in the prescribed format (Annexure - I) within a period of 30 days of the publication of this notice on the website through email (sp-sampling@fssai.gov.in).
4. It may kindly be noted that only such of the comments/suggestions would be taken into consideration as are furnished with supporting scientific evidence.


(Dr. Dinesh Kumar)
Panel Coordinator

Scientific Panel on Methods of Sampling & Analysis
sp-sampling@fssai.gov.in

**MANUAL OF METHODS
OF
ANALYSIS OF FOODS

DAIRY AND DAIRY PRODUCTS**

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Note: The test methods given in the manual are standardized/ validated/ taken from national or international methods or recognized specifications, however it would be the responsibility of the respective testing laboratory to verify the performance of these methods onsite and ensure that it gives proper results before putting these methods in to use.

A. LIQUID MILK

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Preparation of Milk Sample		
Method No.	FSSAI 01.001:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of liquid milk (buffalo milk, cow milk, goat milk, sheep milk, camel milk, mixed milk, standardized milk, full cream milk, recombined milk, toned milk, double toned milk, and skimmed milk).		
Materials and reagents	<ol style="list-style-type: none"> 1. Beakers of various capacity. 2. Water bath. 3. Thermometer. 		
Preparation of reagents	Ammonia solution (Specific Gravity 0.91).		
Sample preparation	<ol style="list-style-type: none"> 1. Samples are received after few days of drawl and contains preservative (0.4% formalin). 2. Warm the sample to 37-40 °C by transferring it to the beaker and keeping it in a water bath maintained at 40-45 °C. 3. Stir slowly for proper homogenization, mix sample thoroughly by pouring back into the bottle, mixing to dislodge any residual fat sticking to the sides and pour it back in the beaker. 4. During mixing do not shake the bottle vigorously. 5. Allow the sample to come to room temperature (26-28 °C) and withdraw immediately for analysis. 6. If small clots or lumps are observed in the sample which cannot be dispersed, a few drops of ammonia solution may be used during homogenization. 		
Inference (Qualitative analysis)	If even after homogenization the sample shows lumps or clots or droplets of oil are visible suggestive of curdling /splitting of milk, the sample should be deemed unfit for analysis and rejected.		
Reference	IS 1479 (Part II):1961 (Reaffirmed year 2018). Methods of test for Dairy Industry -Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Detection of Sucrose (cane sugar) in Milk

Method No.	FSSAI 01.002:2021	Revision No. & Date	0.0
Scope	This method is for the detection of sucrose in milk by Seliwanoff's test		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Resorcinol: Avoid using for a prolonged period, on a broken skin. For external use only; avoid contact with eyes, mouth, and nose. Caution should be exercised in patients with any allergy, children, elderly, during pregnancy and breastfeeding. 		
Principle	<p>For the detection of sucrose in milk, Seliwanoff's test is used which is a test for detection of ketoses such as fructose. Sucrose gives a positive test with Seliwanoff's reagent as it is a disaccharide consisting of fructose and glucose. The dilute hydrochloric acid is used in preparation of Seliwanoff's reagent (resorcinol). The boiling helps in dehydrating the sugar; further keto group more actively attacks resorcinol in comparison to aldehyde group. In case of sucrose, acid leads to its hydrolysis into constituent monosaccharides i.e. glucose and fructose. 5-hydroxymethylfurfural is produced from fructose; a keto sugar. The dehydration product 5-hydroxymethylfurfural condenses with resorcinol forming cherry red colour. Test can be done directly on milk or whey obtained from milk sample.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Water bath. 3. Whatman No. 1 filter paper. 4. Analytical balance (readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Resorcinol. 2. Concentrated hydrochloric acid (Approximately 11.6 N). 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Dilute hydrochloric acid: Take 30 mL concentrated hydrochloric acid and dilute it to 100 mL with distilled water. 		

	2. Resorcinol solution (0.05%): The reagent is prepared by dissolving 0.05 g of resorcinol in 100 mL dilute hydrochloric acid.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Using milk as a medium:</p> <ol style="list-style-type: none"> 1. Take 3 mL milk and 5 mL resorcinol solution in a test tube. 2. Keep the contents in a boiling water bath for 6 min. 3. Cool the tubes immediately after heating under tap water to retard the rate of reaction, which if not done would narrow the colour difference between negative and positive samples. 4. Observe for colour development. <p>Using whey as a medium:</p> <ol style="list-style-type: none"> 1. Take 3 mL whey and 5 mL resorcinol solution in a test tube (quantity of both whey and reagent can be doubled proportionately to get sufficient filtrate.) 2. Mix and filter the contents using Whatman No. 1 filter paper. 3. Keep the filtrate in boiling water bath for 4 min. 4. Cool the tubes immediately after heating under tap water to retard the rate of reaction, which if not done would narrow the colour difference between negative and positive samples. 5. Observe for colour development.
Inference (Qualitative analysis)	<p>Development of red colour indicates adulteration of sucrose in milk. The intensity of red colour increases with increase in the sucrose content in the milk, whereas pure milk remains light in colour. The limit of detection is: 0.1 g/ 100 mL milk (when test is performed in milk) 0.06 g/ 100 mL milk (when test is performed in whey)</p> <p>In case sample is preserved with formaldehyde, adulterated milk will show light pink colour for both milk and whey as a medium of analysis.</p>
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 26-28. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. IS 1479 (Part II): 1961 (Reaffirmed Year: 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Starch in Milk

Method No.	FSSAI 01.003:2021	Revision No. & Date	0.0
Scope	This method is for the detection of starch in milk by Iodine test		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 2. Iodine: It is not compatible with combustibles, strong bases, halogens and ethanol. It reacts violently or explosively with acetylene, acetaldehyde, metal hydrides and metal carbides. 3. Glacial acetic acid: Use with adequate ventilation. Keep away from heat. Do not breathe gas/fumes/ vapor/spray. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, alkalis. 4. Citric acid: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Excessive intake of citric acid may cause erosion of the teeth. Causes respiratory tract irritation. Repeated exposure may cause sensitization dermatitis. 		
Principle	<p>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. The other component, amylopectin, gives a red-purple colour which is much less intense than the amylose. The acidic condition in the reagent mixture accentuates the blue colour, whereas alkali reduces its intensity, the blue colour disappears above a pH of about 9.5. Heating the solution containing starch-iodine complex also destroys the colour although reversibly. Test can be done directly on milk or whey obtained from milk sample after coagulation with citric acid.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Conical flask. 3. Water bath. 4. Whatman No. 1 filter paper. 5. Analytical balance (readability 0.01 g). 		

Materials and Reagents	<ol style="list-style-type: none"> 1. Potassium iodide. 2. Iodine crystals. 3. Citric acid monohydrate. 4. Glacial acetic acid.
Preparation of reagents	<ol style="list-style-type: none"> 1. Iodine solution: Dissolve 2.5 g potassium iodide and 1 g of pure iodine crystals in 100 mL distilled water. Prepare iodine solution at least a day before as iodine dissolves slowly. 2. Acetic acid (10%): Dissolve 10 mL glacial acetic acid in distilled water and make up the volume to 100 mL. 3. Citric acid solution (5%): Dissolve 5 g citric acid monohydrate in distilled water and make up the volume to 100 mL with distilled water.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Using milk as a medium:</p> <ol style="list-style-type: none"> 1. Take 3 mL milk in test tube; bring the milk to boil on a direct flame or on a boiling water bath. 2. Cool the test tube to room temperature (25 ± 3 °C) under tap water. 3. Add a drop of 10% acetic acid in the test tube, and 0.2 mL of iodine solution. 4. Mix the content and observe colour. <p>Using whey as a medium:</p> <ol style="list-style-type: none"> 1. Take 20 mL milk in a conical flask, bring the milk to boil on a direct flame or on a boiling water bath. 2. Add 5% citric drop wise till visible coagulation (approximate consumption of citric acid required would be 1.5-2 mL). 3. Filter the content using Whatman No. 1 filter paper; let the filtrate cool to room temperature. 4. Take 3 mL filtrate in another test tube and add 0.1 mL of iodine solution. 5. Mix the contents and observe colour.
Inference (Qualitative analysis)	<p>Blue/dark blue colour formation indicates adulteration of milk with starch, whereas pure milk remains yellow due to colour of iodine. The limit of detection is:</p> <p>0.02 g/ 100 mL milk (when test is performed in milk) 0.01 g/ 100 mL milk (when test is performed in whey)</p> <p>In case sample is preserved with formaldehyde, adulterated milk will show same blue colour for milk and dark blue colour for whey.</p>
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 31-32. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat).

	<p>2. IS 1479 (Part I):2016. Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi.</p> <p>3. Moulay, S. (2013). Molecular iodine/polymer complexes. Journal of Polymer Engineering, 33(5), 389-443.</p>
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Urea in Milk

Method No.	FSSAI 01.004:2021	Revision No. & Date	0.0
Scope	This method is for the detection of urea in milk by p-dimethylaminobenzaldehyde (DMAB) test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Methanol: It is a highly flammable liquid and vapour. Keep away from heat, sparks, open flames, hot surfaces. Perform all operations under a fume hood. 2. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 3. p-Dimethylaminobenzaldehyde: May be harmful if absorbed through skin. May cause skin irritation. May cause irritation in eyes. May be harmful if swallowed Good general ventilation should be sufficient to control worker exposure to airborne contaminants. 		
Principle	This method is based on the principle that urea forms a yellow-coloured complex with p-dimethylaminobenzaldehyde (DMAB) reagent in low acidic alcoholic solution at room temperature (25 ± 3 °C). The intensity of colour can be measured at 440 nm. The colour developed is in proportion to urea content in the sample.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.01 g). 2. Test tube. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. p-Dimethylaminobenzaldehyde reagent (DMAB). 2. Methanol. 3. Concentrated hydrochloric acid (Approximately 11.6 N). 		
Preparation of reagents	p-Dimethylaminobenzaldehyde reagent (DMAB): The reagent is prepared by dissolving 1.6 g of p-dimethylaminobenzaldehyde in methanol subsequently adding 10 mL of concentrated hydrochloric acid and making volume to 100 mL with methanol.		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take 5 mL milk in a test tube, add 5 mL DMAB reagent. 		


	2. Mix the contents and observe for colour.
Inference (Qualitative analysis)	<p>Formation of distinct yellow colour indicates the presence of added urea in milk sample, whereas pure milk shows light yellow colour due to natural urea. The limit of detection is 0.2 g/ 100 mL milk.</p> <p>This method is a preliminary method for screening purpose. Positive samples are required to be confirmed by quantitative method (Method No. FSSAI 01.005:2021).</p>
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 23-24. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. IS 1479 (Part I):2016. Methods of test for Dairy Industry, Part – I Rapid examination of Milk. Bureau of Indian Standards, New Delhi. 3. Bector, B.S., Ram, M. & Singhal, O.P. (1998). Rapid platform test for the detection /determination of added urea in milk. Indian Dairyman, 50(4): 59-62
Approved by	Scientific Panel on Methods of Sampling and Analysis

Quantitative Estimation of Total Urea Content in Milk

Method No.	FSSAI 01.005:2021	Revision No. & Date	0.0
Scope	This method is for the quantitative estimation of total urea in milk by dimethylaminobenzaldehyde (DMAB) method.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. Urea: Avoid contact and inhalation. Do not get in eyes, on skin, on clothing. Wash thoroughly after handling. Keep in a tightly closed container, stored in a cooled, dry, ventilated area. Urea reacts with calcium hypochlorite or sodium hypochlorite to form the explosive nitrogen trichloride. It is incompatible with sodium nitrite, gallium perchlorate, strong oxidizing agents (permanganate, dichromate, nitrate, chlorine). 3. p-Dimethylaminobenzaldehyde: Good general ventilation should be sufficient to control worker exposure to airborne contaminants. 4. Trichloroacetic acid: Trichloroacetic acid is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Trichloroacetic acid can irritate the nose and throat. Trichloroacetic acid can irritate the lungs causing coughing and/or shortness of breath. 5. Potassium dihydrogen orthophosphate: Avoid contact with skin and eyes. Hygiene measures: Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Storage conditions: Store in a well-ventilated place. Keep container tightly closed. 		

	6. Dipotassium monohydrogen orthophosphate: On eye and skin contact, rinse and wash off immediately with plenty of water, also under the eyelids, for at least 15 min. Obtain medical attention. On inhalation, move to fresh air; If breathing is difficult, provide oxygen.
Principle	This method is based on the principle that urea forms a yellow-coloured complex with p-dimethylaminobenzaldehyde (DMAB) reagent in low acidic alcoholic solution at room temperature (25 ± 3 °C). The intensity of colour is measured at 425 nm.
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Spectrophotometer: Instrument with maximum band width 1 nm at 425 nm and 1 cm path length. 2. Whatman filter paper: Grade 42. 3. Funnels. 4. Test tubes. 5. Volumetric flasks: 25 mL, 100 mL and 1 L. 6. Analytical balance (readability 0.01 g).
Materials and reagents	<ol style="list-style-type: none"> 1. p-Dimethylaminobenzaldehyde (DMAB). 2. Ethyl alcohol. 3. Concentrated hydrochloric acid (Approximately 11.6 N). 4. Trichloroacetic acid (TCA). 5. Urea. 6. Anhydrous potassium dihydrogen orthophosphate. 7. Anhydrous dipotassium monohydrogen orthophosphate.
Preparation of reagents	<ol style="list-style-type: none"> 1. p-Dimethylaminobenzaldehyde (DMAB) solution: Dissolve 1.6 g DMAB in 100 mL ethyl alcohol and add 10 mL concentrated hydrochloric acid. The reagent is stable for 1 month. Prepare new standard curve with each new batch of reagent. 2. Phosphate buffer pH 7.0 (0.05 M): Dissolve 3.154 g anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) and 4.672 g anhydrous dipotassium monohydrogen orthophosphate (K_2HPO_4) separately in 100 mL of distilled water. Combine both the solutions and dilute to 1 L with water. 3. Trichloroacetic acid (TCA) 24%, w/v (freshly prepared): 24.0 g TCA is dissolved in distilled water and the volume is made up to 100 mL. 4. Diluting reagent: Equal volumes of 24% TCA and phosphate buffer (pH 7.0) are mixed to make the diluting reagent. 5. Urea Standard Solution (1 mg/mL): Weigh 25 mg of urea and dissolve in phosphate buffer of pH 7.0 and make up the volume to 25 mL with phosphate buffer. Store at less than 24 °C. The reagent is stable for 1 week.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis:	a) Preparation of standard curve

	<ol style="list-style-type: none"> 1. Take 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.5 mL of urea standard solution in 20 x 150 mm (25) mL test tube and make up the volume to 5 mL by adding 4.9, 4.8, 4.6, 4.4, 4.2, 4.0 and 3.5 mL diluting reagent. The concentration of the solutions will be 20, 40, 80, 120, 160, 200 and 300 µg/mL. Add 5 mL DMAB solution to each to develop the yellow colour. Prepare reagent blank of 5 mL diluting reagent and 5 mL DMAB solution. 2. Shake tubes thoroughly and let stand for 10 min. Read absorbance in 1 cm cell at 425 nm with reagent blank at zero. 3. Plot absorbance against the concentration of urea; plot should be straight line ($R^2 > 0.99$) <p>b) Extraction</p> <ol style="list-style-type: none"> 1. 10 mL of milk sample is mixed with 10 mL of TCA to precipitate the proteins and filtered using Whatman 42 filter paper. 2. 5 mL of filtrate is then treated with 5 mL of DMAB reagent to develop the colour. 3. Blank is prepared by taking 5 mL of diluting reagent and treating it with 5 mL of DMAB reagent. <p>c) Checking of Optical Density</p> <ol style="list-style-type: none"> 1. The optical density of the yellow color is measured at 425 nm.
<p>Calculation with units of expressions</p>	<p>From standard curve, the amount of urea in milk is calculated. Read from the graph the concentration of urea (µg) corresponding to absorbance of sample.</p> <p>Consider the concentration from the standard curve for urea is = Y µg</p> <p>So, 1 mL of filtrate from sample has = Y µg urea</p> <p>10 mL of filtrate (milk + TCA) has = Y * 10 µg urea</p> <p>i. e. 5 mL milk has = Y * 10 µg urea</p> <p>100 mL of milk sample has = $\frac{Y * 10 * 100}{5}$ µg urea per 100 mL of milk</p> <p style="text-align: center;">$= \frac{Y}{5}$ mg urea per 100 mL of milk</p>
<p>Reference</p>	<ol style="list-style-type: none"> 1. IS 1479 (Part I):2016. Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi. 2. Bector, B.S., Ram, M. & Singhal, O.P. (1998). Rapid platform test for the detection /determination of added urea in milk. Indian Dairyman, 50(4): 59-62
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Detection of Ammonium Salts in Milk		
Method No.	FSSAI 01.006:2021	Revision No. & Date	0.0
Scope	This method is for the detection of ammonium salts in milk by Nessler's test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Mercuric chloride: Reacts violently with strong oxidizers. Avoid formation of dust, perform all operations under a fume hood. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 3. Potassium iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 4. Citric acid: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Excessive intake of citric acid may cause erosion of the teeth. Causes respiratory tract irritation. Repeated exposure may cause sensitization dermatitis. 		
Principle	Salts like ammonium sulfate, ammonium nitrate and ammonium dihydrogen orthophosphate are added in milk to raise the SNF. Ammonium sulphate is also a nitrogen containing molecule, which if used as adulterant can also raise nitrogen content if determined by Kjeldahl method. Nessler's test is one of the classical methods for qualitative and quantitative analysis of ammonia and ammonium ions. Nessler's reagent is an alkaline solution of potassium mercuric iodide (K_2HgI_4). On reaction with ammonium ion Nessler's reagent produces a yellowish brown colour. The intensity of the colour is directly proportional to the amount of ammonia/ammonium ion present.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Conical flask. 3. Water bath. 4. Whatman No. 1 filter paper. 5. Analytical balance (readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Mercuric chloride. 2. Sodium hydroxide. 		

	<ol style="list-style-type: none"> 3. Potassium iodide. 4. Citric acid.
Preparation of reagents	<ol style="list-style-type: none"> 1. Nessler's reagent: Dissolve the following chemicals separately. <ol style="list-style-type: none"> a) 8.0 g of mercuric chloride in 150 mL distilled water. b) 60.0 g of sodium hydroxide in 150 mL distilled water. c) 16.0 g of potassium iodide in 150 mL distilled water. Add reagent 'a' to reagent 'b' and mix well. To this mixture, add reagent 'c', mix and dilute the contents to 500 mL. Leave this solution undisturbed and decant the clear upper layer of the solution. Store in a stoppered amber glass bottle. Note: Alternatively, commercially available (readymade) Nessler's reagent can also be used. 2. Citric acid solution (5%): Dissolve 5 g citric acid monohydrate in distilled water and make up the volume to 100 mL with distilled water.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Take 20 mL milk in a conical flask. 2. Warm the milk to 70-80 °C either on direct flame or water bath. 3. Add 5% citric acid solution drop wise in the milk with gentle stirring till visible coagulation occurs. 4. Flask, if stirred vigorously will result in fine curd particles and which may impact colour observation. 5. Filter the content using Whatman No. 1 filter paper. 6. Take 5 mL of filtrate into a test tube, add 0.4 mL of Nessler's reagent. Observe for colour without shaking the test tube.
Inference (Qualitative analysis)	Milk adulterated with ammonium salts shows instant development of orange colour, whereas pale yellow colour indicates unadulterated milk. In case sample is preserved with formaldehyde, adulterated milk will show muddy brown colour. Limit of detection is 0.02 g/ 100 mL milk.
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 25-26. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi. 3. Sharma, R.; Rajput, Y.S. & Naik, N.L. (2012). Detection of adulterants in milk – a laboratory manual. NDRI Publication No. 88/2012, NDRI, Karnal, page 49-51).
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Sulphates in Milk

Method No.	FSSAI 01.007:2021	Revision No. & Date	0.0
Scope	This method is for the detection of sulphates in milk by barium chloride test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Barium chloride: Wash thoroughly after handling. Wash thoroughly after handling. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Do not ingest or inhale. 2. Lactic acid: Ensure adequate ventilation. Wear personal protective equipment. Do not get in eyes, on skin, or on clothing. Avoid ingestion and inhalation. 		
Principle	<p>This method is based on the reaction of barium chloride with sulphates. The solution of barium chloride reacts with sulphate in milk (protein free filtrate) to give barium sulphate. Using this test, presence of added sulphate like ammonium sulphate, sodium sulphate, zinc sulphate, magnesium sulphate, etc. to milk can be confirmed by observing milky-white precipitates.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Conical flask. 3. Water bath. 4. Whatman No. 42 filter paper. 5. Analytical balance (Readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Barium chloride. 2. Lactic acid (commercially available 85% assay). 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Barium chloride solution (5%): Dissolve 5.0 g barium chloride in distilled water and make up the final volume to 100 mL. 2. Lactic acid solution (5%): Take 5 mL of commercially available lactic acid in 100 mL volumetric flask and make up the volume with distilled water. 		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation		
Method of analysis	<ol style="list-style-type: none"> 1. Take 20 mL milk in a conical flask/test tube bring it to boil on direct flame, add 2 mL of 5% lactic acid in hot milk and filter the contents using Whatman No. 42 filter paper. 		

	2. Take 2 mL filtrate in a separate test tube, add 0.2 mL of 5% barium chloride and observe for turbidity development.
Inference (Qualitative analysis)	Formation of turbidity after addition of barium chloride solution indicates adulteration of milk with sulphate. The limit of detection is 0.015 g/ 100 mL milk.
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 36-37. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. Singh, P., & Gandhi, N. (2015). Milk preservatives and adulterants: processing, regulatory and safety issues. Food Reviews International, 31(3), 236-261. 3. Sharma, R.; Rajput, Y.S. & Naik, N.L. (2012). Detection of adulterants in milk – a laboratory manual. NDRI Publication No. 88/2012, NDRI, Karnal, page 20-21).
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Glucose in Milk

Method No.	FSSAI 01.008:2021	Revision No. & Date	0.0
Scope	This method is for the detection of glucose in milk by Barfoed's test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Copper acetate: Wash thoroughly after handling. Wash thoroughly after handling. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. Store in a cool, dry, well-ventilated area away from incompatible substances. 2. Lactic acid: Ensure adequate ventilation. Wear personal protective equipment. Do not get in eyes, on skin, or on clothing. Avoid ingestion and inhalation. 		
Principle	This test is based on the reaction of glucose (a monosachharide) with Barfoed's reagent. By means of the Barfoed's reaction, it is possible to differentiate reducing monosaccharaides from reducing disaccharides (lactose in case of milk) as monosaccharaides can reduce copper fast enough in comparison to disaccharides. The formation of green precipitate is a positive test for reducing monosaccharides.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Water bath. 3. Analytical balance (Readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Copper acetate. 2. Lactic acid (commercially available 85% assay). 		
Preparation of reagents	Barfoed's reagent: Dissolve 13.3 g of copper acetate in distilled water, subsequently add 2.0 mL of lactic acid and make up the total volume to 200 mL.		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take 1 mL milk in a test tube, add 2 mL Barfoed's reagent. 2. Keep the test tube in boiling water bath for 6 min. 3. Cool the test tube to room temperature (25 ± 3 °C) under tap water. 4. Observe for colour development. 		
Inference (Qualitative analysis)	Development of green colour indicates presence of glucose in milk. The limit of detection is 0.1 g/100 mL milk. In case sample is preserved with formaldehyde, adulterated milk will give bluish-green colour.		

Reference	Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 28-29. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat).
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Sodium Chloride in Milk

Method No.	FSSAI 01.009:2021	Revision No. & Date	0.0
Scope	This method is for the detection of sodium chloride in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Silver nitrate: It is corrosive. It is not combustible, but it is a strong oxidizer that enhances the combustion of other substances. 2. Potassium chromate: It is a strong oxidizing agent. It is a carcinogen and mutagen and should be handled with extreme caution. 		
Principle	The presence of extraneously added sodium chloride in milk can be detected by silver nitrate and potassium chromate reagent. In presence of sodium chloride in milk, the silver nitrate does not react with potassium chromate to form brown precipitate of silver chromate.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Glass test tube. 2. 100 mL volumetric flask. 3. Analytical balance (Readability 0.01 g). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Silver nitrate. 2. Potassium chromate. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Silver nitrate (AgNO_3) solution: 0.1 N, aqueous Dissolve 1.6987 g of AgNO_3 and make up the volume to 100 mL with distilled water. Standardize the prepared silver nitrate with sodium chloride. 2. Potassium chromate (K_2CrO_4) solution: 10% (w/v) aqueous.: Dissolve 10 g of potassium chromate in distilled water and make up the total volume to 100 mL. 		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take 5.0 mL of milk sample and add 2.0 mL of 0.1 N silver nitrate solution 2. Mix the contents thoroughly and add 0.5 mL of 10% potassium chromate solution and observe the colour. 		
Inference (Qualitative analysis)	The appearance of yellow colour indicates presence of added chloride in milk, whereas chocolate brown precipitate indicates the absence of added chloride in milk		
Reference	<ol style="list-style-type: none"> 1. Pearson's Composition and Analysis of Foods, 9th edn, 1991 – Modified Mohr method, page 14. 2. FSSAI Order. (2018). Revised procedure for detection of sodium chloride in milk. File No. 1-90/FSSAI/SP (MS&A)/2009 dated 21st 		

	March, 2018. Food Safety Standards Authority of India, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Method for Detection of Presence of Foreign Fat in Milk

Method No.	FSSAI 01.010:2021	Revision No. & Date	0.0
Scope	This method is for the detection of presence of foreign fat in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Iso-amyl alcohol: It is a highly flammable liquid. Keep away from heat/sparks/open flames/hot surfaces. Perform all operations under a fume hood. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 		
Principle	<p>In the following test, milk fat is isolated from given milk sample and is subjected to butyro-refractometer (BR) reading. Since, most of vegetable fat/oils have higher B.R. compared to milk fat, any increase in B.R. reading above reference value of milk fat indicates adulteration of milk with vegetable/refined oil. For isolation of milk fat from milk, modified Gerber butyrometer can be used where both ends of the butyrometer are open. Stem side opening of the butyrometer (which is generally closed) is closed with a good quality removable silicon stopper. After the milk fat test, silicon stopper is removed and milk fat is removed with the help of a syringe and same is subjected to BR at 40 °C. Since Gerber sulphuric acid causes some hydrolysis of fatty acids/triglycerides, the B.R is multiplied by a factor to obtain corrected B.R. The fat in suspected milk sample can also be isolated by solvent extraction method in which case correction of B.R. is not required.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Modified Gerber Butyrometer (with both ends open) 2. Pipette: 10.75 ± 0.03 mL for milk at 27 °C. 3. Automatic measure or tilt measure for dispensing Gerber sulphuric acid: 10 ± 0.25 mL at 27 °C. 		

	<ol style="list-style-type: none"> 4. Automatic measure or tilt measure for dispensing iso-amyl alcohol: 1 ± 0.05 mL at 27 °C. 5. Butyro-refractometer (B.R.). 6. Centrifuge. 7. Analytical balance (Readability 0.01 g).
Materials and reagents	<ol style="list-style-type: none"> 1. Concentrated sulphuric acid (Approximately 36.8 N). 2. Iso amyl alcohol (furfural free). It should have density between 0.807 to 0.809 g/mL at 27 °C. 3. Diethyl ether.
Preparation of reagents	<ol style="list-style-type: none"> 1. Gerber Sulphuric acid: Take required volume of water in a pyrex flask (generally 100 mL of water is required for 900 mL of concentrated sulphuric acid) kept in a basin of ice-cold water. Carefully add the concentrated sulphuric acid in small quantities at a time keeping the container sufficiently cold and mix gently. After cooling the flaks, check the specific gravity of Gerber acid with hydrometer and if necessary, adjust the Gerber acid to the correct specific gravity with addition of water or acid taking same precautions as before till specific gravity is in the range of 1.807 to 1.812 g/mL at 27 °C (or 1.815 to 1.820 g/mL at 20 °C). Store the prepared acid in a glass stoppered bottle to avoid absorpion of water.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation
Method of analysis	<p>Modified Gerber Method:</p> <ol style="list-style-type: none"> 1. Isolate the fat from milk by Gerber method using specially designed milk butyrometer, which is open at both ends; close the stem side opening with a good quality acid resistant silicon stopper. 2. Add 10 mL of Gerber sulphuric acid, 10.75 mL milk and 1 mL iso-amyl alcohol; close the neck side with lock stopper; mix the contents and centrifuge at 1100 rpm (approximately 300 g) for 5 min to get a clear fat column. 3. Remove the silicon stopper from the stem side and take out the fat from the stem of the butyrometer using a capillary or a syringe. 4. For taking BR. reading of the milk fat, clean the prism of the butyro-refractometer with diethyl ether and allow the ether to evaporate to dryness. 5. Maintain the temperature of the prism at 40 °C by circulating water using a thermostatically controlled water-bath or thermostatically controlled automatic butyro- refractometer. 6. Calibrate the butyro-refractometer by applying standard liquid solution of known BR reading. 7. Again, clean the prism with diethyl ether; apply 1-2 drops of clear,

	<p>extracted fat between the prism.</p> <p>8. Wait for 2 min before taking the reading so that the sample should attain temperature of 40 °C.</p> <p>9. A correction of 0.55 is added to the observed B.R. reading for each degree above 40 °C or subtracted for each degree below 40 °C to get corrected B.R. reading of the sample.</p> <p>Solvent Extraction Method:</p> <ol style="list-style-type: none"> 1. Extract fat from the milk sample by Rose-Gottlieb method. 2. The B.R. reading at 40 °C of the extracted fat and interpret the results. <p>Cream Extraction Method:</p> <ol style="list-style-type: none"> 1. Separate the milk fat from the milk sample by centrifugation (5000 g, 10 min, 4 °C). 2. Carefully remove the cream plug and collect the cream in an aluminium dish. 3. Heat the dish over burner till ghee formation takes place. 4. Filter the ghee residues and take the BR reading of ghee and interpret the results. <p>Note:</p> <p>(a) If fat is extracted using modified Gerber method, correction factor is required.</p> <p>(b) Result can be interpreted directly from the B.R. reading if fat is extracted using other method. Further, check for the presence of extraneous fat can be done by checking the fatty acid profile of the extracted fat by GLC.</p>
Calculation with units of expression	<p>Calculate the corrected B.R. reading of isolated fat as follows: Corrected B.R. = Observed B.R. × 1.08 (correction factor is required only in case fat is extracted with modified Gerber method)</p>
Inference (Qualitative analysis)	<p>If the B.R. reading differs from the prescribed limit specified for states / Union Territory (as indicated in Clause 2.1.8 of FSS (Food Products Standards and Food Additives), 2011) presence of foreign fat in the milk may be suspected.</p>
Reference	<ol style="list-style-type: none"> 1. IS 1479 (Part I):2016. Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi. 2. Arora, K.L.; Lal, D.; Seth, R. & Ram, J. (1996). Platform test for detection of refined mustard oil adulteration in milk. Indian J. Dairy Sci., 49 (10): 721-723. 3. Lal, D.; Seth, R.; Arora, K.L. & Ram, J. (1998). Detection of vegetable oils in milk. Indian Dairyman, 50 (7): 17-18.
Approved by	<p>Scientific Panel on Methods of Sampling and Analysis</p>

Detection of Nitrate in Milk

Method No.	FSSAI 01.011:2021	Revision No. & Date	0.0
Scope	This method is for the detection of nitrate in milk by diphenylamine test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Glacial acetic acid: Use with adequate ventilation. Keep away from heat. Do not breathe gas/fumes/ vapor/spray. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, alkalis. 3. Diphenylamine: Avoid contact with skin and eyes. Do not breathe vapours. Provide good ventilation in process area to prevent formation of vapours. 		
Principle	The test consists of adding a solution of diphenylamine prepared in sulphuric acid to milk. In the presence of nitrates, diphenylamine is oxidized, giving a blue coloration. Hence, development of blue colour indicates the presence of nitrate.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Conical flask. 3. Water bath. 4. Whatman No. 1 filter paper. 5. Analytical balance (Readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Diphenylamine. 2. Concentrated sulphuric acid (Approximately 36.8 N). 3. Glacial acetic acid. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Diphenylamine solution: The reagent is prepared by dissolving 0.085 g diphenylamine in 50 mL distilled water and gradually 450 mL of concentrated sulphuric acid is added with constant stirring. During preparation of reagent the content is kept cool by dipping in cold water. Diphenyl amine solution should be prepared freshly and shall be colourless. 		

	2. Acetic acid (10%): Take 10 mL glacial acetic acid in 100 mL volumetric flask and make up the volume with distilled water.
Method of analysis	<ol style="list-style-type: none"> 1. Take 20 mL milk in a conical flask; warm the milk to 70-80 °C either on direct flame or boiling water bath. 2. Add 10% acetic acid drop wise in the milk with gentle stirring till visible coagulation. 3. Filter the contents using Whatman No. 1 filter paper. 4. Take 2 mL diphenylamine solution in a test tube, add 1 mL filtrate in test tube containing diphenylamine solution. 5. Observe for ring formation at the junction of two solutions.
Inference (Qualitative analysis)	Formation of blue ring at the junction of two solutions indicates adulteration of milk with nitrate or surface water. The limit of detection is 0.002 g/100 mL milk.
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 34-36. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. Sharma, R.; Rajput, Y.S. & Naik, N.L. (2012). Detection of adulterants in milk – a laboratory manual. NDRI Publication No. 88/2012, NDRI, Karnal, page 47-48.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Neutralizers in Milk

Method No.	FSSAI 01.012:2021	Revision No. & Date	0.0
Scope	This method is for the detection of neutralizers in milk by phenol red test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <p>1. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours.</p>		
Principle	<p>This reaction is based on the pH of milk, wherein phenol red is used as a pH indicator. It shows colour transition from yellow to red over the pH range 6.8 to 8.2. At pH value greater than 8.2 phenol red turns a bright pink. Neutralization of milk causes increase in the pH which can be detected using this indicator.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.01 g) 2. Test tube 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Phenol red 2. Ethanol 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Phenol red solution: Dissolve 0.05 g of phenol red in 20 mL ethanol and make up the volume up to 100 mL using distilled water. 		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation		
Method of analysis	<ol style="list-style-type: none"> 1. Take 4 mL milk in a test tube, add 1 mL phenol red solution. 2. Mix the contents and observe for colour. 		
Inference (Qualitative analysis)	<p>Development of pinkish orange colour indicates neutralizer adulterated milk, whereas unadulterated sample remains yellow. The limit of detection is as follows:</p> <p>Sodium hydroxide: 0.04 g/100 mL milk Na_2CO_3: 0.08 g/ 100 mL milk NaHCO_3: 0.2 g/ 100 mL milk</p> <p>In case sample is preserved with formaldehyde, adulterated milk will show pinkish orange colour with reduced intensity.</p>		
Reference	Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 42. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat).		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Method for Detection of Hypochlorite and Chloramines in Milk

Method No.	FSSAI 01.013:2021	Revision No. & Date	0.0
Scope	This method is for detection of hypochlorite and chloramines in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Stannous chloride: It is non-combustible; substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. Thermal decomposition can lead to release of irritating gases or vapours. 2. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 3. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 		
Principle	In this test, yellowish fluorescence is produced due to the presence of chlorate (potassium or sodium chlorate) in the hypochlorite solution and is directly proportional to the amount of chlorate present. Stannous chloride in the reaction acts as reducing agent.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Centrifuge. 2. Tubes for centrifuge: 12.5 mL capacity. 3. UV lamp. 4. Analytical balance (Readability 0.01 g). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$). 2. Potassium iodide. 3. Concentrated hydrochloric acid (Approximately 11.6 N). 		

	<ol style="list-style-type: none"> 4. Starch. 5. Concentrated sulphuric acid (Approximately 36.8 N). 																				
Preparation of reagents	<ol style="list-style-type: none"> 1. Stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) solution: 0.025%, (w/v) in 73.5% sulphuric acid (prepared by mixing three volumes of concentrated sulphuric acid and one volume of distilled water). 2. Potassium iodide (KI) solution: Prepare fresh by dissolving 7 g of KI in 100 mL water. 3. Dilute hydrochloric acid: To 200 mL of water, add 100 mL concentrated hydrochloric acid. 4. Starch solution: Boil 1 g starch in 100 mL water. Cool before using. 																				
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.																				
Method of analysis	<p>Method 1. Detection of Hypochlorite:</p> <ol style="list-style-type: none"> 1. Cool about 3 mL of milk sample in a test tube in a freezing mixture of ice and salt to 2-5 °C. 2. In another test tube, take an equal volume of the stannous chloride solution and similarly cool and add to milk. Shake the tube whilst in freezing mixture and hold for 3 min. 3. Place the mixture in a centrifuge tube and centrifuge for 3 min at 2500 rpm (approximately 1012 g). 4. A yellow-green colour is produced in the presence of hypochlorite. Alternatively, after centrifuging, examine the tube in ultraviolet light for the presence of any yellow fluorescence. <p>Method 2. Detection of Hypochlorite and Chloramines:</p> <ol style="list-style-type: none"> 1. To 5 mL of sample in a test tube add 1.5 mL of potassium iodide solution, mix thoroughly by shaking and note the colour of milk. 2. If unaltered, add 4 mL of dilute hydrochloric acid, mix thoroughly with a glass rod flattened at one end and note the colour of curd. 3. Subsequently, place the tube in a water bath previously heated to 85 °C and allow it to remain for 10 min. The curd will rise to the surface. Cool the tube rapidly by placing in cold water. Note the colour of the curd and the liquid. 4. Next add 0.5 to 1.0 mL of starch solution to the liquid below curd and note the colour. 																				
Inference (Qualitative analysis)	<p>Table.1. Reactions with various tests to detect residual chlorine in milk.</p> <table border="1"> <thead> <tr> <th rowspan="2">Test No</th> <th colspan="6">Concentration of available chlorine</th> </tr> <tr> <th>1:1000</th> <th>1:2000</th> <th>1:5000</th> <th>1:10000</th> <th>1:25000</th> <th>1:50000</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>Yellowish brown</td> <td>Deep yellow</td> <td>Pale yellow</td> <td>-</td> <td>-</td> <td>-</td> </tr> </tbody> </table>	Test No	Concentration of available chlorine						1:1000	1:2000	1:5000	1:10000	1:25000	1:50000	A	Yellowish brown	Deep yellow	Pale yellow	-	-	-
Test No	Concentration of available chlorine																				
	1:1000	1:2000	1:5000	1:10000	1:25000	1:50000															
A	Yellowish brown	Deep yellow	Pale yellow	-	-	-															

	B	Yellowish brown	Deep yellow	Light yellow	-	-	-
	C	Yellowish brown	Deep yellow	Yellow	Yellow	Pale yellow	Yellowish
	D	Blue purple	Blue purple	Blue, dark purple	Dark purple	Red purple	Pale red-purple
	<p>* Indicates the step number followed in Method 2 of the above procedure. Note: The method is not reliable in the presence of more than 2.5 ppm of copper.</p>						
Reference	<p>1. IS 1479 (Part I): 2016. Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi.</p> <p>2. AOAC 922.08. 21st Edn. (2019). Hypochlorites and Chloramines in milk. AOAC International, USA.</p>						
Approved by	Scientific Panel on Methods of Sampling and Analysis						

Detection of Quaternary Ammonium Compounds in Milk

Method No.	FSSAI 01.014:2021	Revision No. & Date	0.0
Scope	This method is for the detection of Quaternary Ammonium Compounds (QAC) in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Tetrachloroethane: It is a colorless, volatile, non-flammable, liquid, chlorinated hydrocarbon with an ether-like odor that may emit toxic fumes of phosgene when exposed to sunlight or flames. 3. Acetone: It is a flammable liquid and dangerous fire hazard. It reacts with strong oxidizing agents (such as perchlorates, chlorine, bromine, fluorine) to form explosive peroxides. Perform all operations under a fume hood. 4. Citric acid: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Excessive intake of citric acid may cause erosion of the teeth. Causes respiratory tract irritation. Repeated exposure may cause sensitization dermatitis. 		
Principle	The amount of QAC can be approximately determined by titration with a standard anionic detergent solution. QAC may be present in milk due to some residual detergent solution remaining after bottle washing. The following method detects about 5 mg / kg in milk.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. 100 mL volumetric flask. 2. Centrifuge tube. 3. Analytical balance (Readability 0.01 g). 		
Materials/reagents	<ol style="list-style-type: none"> 1. Eosin. 2. Acetone. 3. Tetrachloroethane. 4. Citric acid. 5. Sodium hydroxide. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Indicator solution: Prepare a stock solution by dissolving 0.05 g eosin in 100 mL acetone. Shake 10 mL of stock solution with 90 mL of tetrachloroethane and 1 g citric acid and filter before use. 		

	<ol style="list-style-type: none"> 2. Sodium hydroxide solution (50%): Dissolve 50 g sodium hydroxide in 100 mL water. 3. Buffer: Dissolve 25 g citric acid in 100 mL water and adjust to pH 3.5 with 50% sodium hydroxide solution (approximately 15 mL required).
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. To a centrifuge tube add 1 mL milk, 5 mL water, 1 mL indicator solution and 0.2 mL buffer and shake hard for 10 sec. 2. Centrifuge for 5 min at 3200 rpm (approximately 1660 g). 3. Observe the colour in bottom layer.
Inference (Qualitative analysis)	QAC present in the sample is indicated by the formation of red or pink colouration. In bottom layer If the colour is deep pink or red, the amount of QAC can be approximately determined by titration with a standard anionic detergent solution. Samples containing about 1 mg/kg of QAC show a faint pink colour.
Reference	Pearson's Composition and Analysis of Foods 9 th edn 1991, page 548.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Anionic Detergents in Milk

Method No.	FSSAI 01.015:2021	Revision No. & Date	0.0
Scope	This method is for the detection of detergents in milk by methylene blue test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Methanol: It is a highly flammable liquid and vapour. Keep away from heat, sparks, open flames, hot surfaces. Perform all operations under a fume hood. 2. Chloroform: Decomposes slowly on exposure to light and air to release toxic and corrosive vapours like phosgene, chlorine etc. It reacts strongly with strong oxidizers and forms toxic vapours. Keep away from heat and strong oxidizing agents. 		
Principle	<p>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. Detergents can be anionic, cationic and non-ionic, among which anionic types are the most commonly used. Methylene blue is cationic dye which forms complex with anionic detergents. It is normally water-soluble compound; however, it shows affinity for anionic detergents, if they are present. In this method, detergents are first extracted in chloroform and then methylene blue solution is added. In presence of detergent, blue colour is developed in chloroform layer of the sample, whereas blue colour is observed in milk layer in control (pure milk). Chloroform is heavier (density 1.49 g/ mL) than milk (density ~1.030 g/ mL), and hence settles at the bottom. This implies that observation of blue colour in the bottom layer indicates presence of detergents.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Whatman No. 1 filter paper. 3. Analytical balance (Readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Methanol. 2. Methylene blue. 3. Chloroform. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Methylene blue solution: 12.5 mg methylene blue is dissolved in 100 mL of distilled water. Protect the solution from direct sunlight. 		

Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Take 2.5 mL of suspected milk sample in a test tube and add 7.5 mL methanol, filter the contents through Whatman No. 1 filter paper. 2. Take 2 mL filtrate in a test tube, add 2 mL of methylene blue solution and shake well. 3. Subsequently, add 4 mL chloroform and shake well again. Allow the chloroform layer to separate. Compare the colour extracted in the chloroform layer in suspected milk with that for pure milk.
Inference (Qualitative analysis)	If the methylene blue colour extracted from a suspected sample into the chloroform layer is darker than that extracted from pure milk sample, it indicates the presence of detergent in milk. The limit of detection is 0.02 g/100 mL milk.
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 22-23. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. Rajput, Y.S.; Sharma, R. & Kaur, S. (2006) A kit for detection of detergent in milk. Indian Patent Office File No. 1970/Del/2006. Indian Patent Grant No. 264661 dated 14.01.2015.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Formaldehyde in Milk

Method No.	FSSAI 01.016:2021	Revision No. & Date	0.0
Scope	This method is for the detection of presence of formaldehyde in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 3. Ferric chloride: It is a corrosive chemical. Substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. It can react with metals to form flammable and potentially explosive hydrogen gas. 		
Principle	<p>Formaldehyde (HCHO) is the simplest aldehyde. Exposure to formaldehyde is reported to be extremely harmful to human health. Formaldehyde manifests toxicity in humans, acute exposure can cause pain, dermatitis, nausea, arrhythmia, coma and renal failure. Chronic exposure increases the risk of cancer. Formaldehyde is permitted as preservative only for analytical purposes in milk and milk product sample; though its presence marketable dairy products is illegal. Two methods can be used for the detection of presence of formaldehyde in milk.</p> <p>In the Hehner test for detection of formaldehyde in milk, concentrated sulphuric acid and ferric chloride is used. The test is an aldehyde-oxidation reaction of an aromatic amine. Ferric chloride acts as oxidising agent for formaldehyde. The formaldehyde reaction depends on the presence of the tryptophan in the protein molecule. The violet colour develops as a result of the reaction of oxidised formaldehyde with tryptophan. The intensity of</p>		

	<p>the reaction with different proteins varies in direct proportion to the amount of tryptophan present in the protein molecule.</p> <p>In Leech test for detection of formaldehyde, concentrated hydrochloric acid is used in place of sulphuric acid (which is used in Hehner test). The contents are heated to enhance oxidation and resultant colour development.</p>
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Water bath. 3. Hot plate or burner. 4. Analytical balance (Readability 0.01 g).
Materials and Reagents	<ol style="list-style-type: none"> 1. Ferric chloride. 2. Concentrated sulphuric acid (Approximately 36.8 N). 3. Concentrated hydrochloric acid (Approximately 11.6 N).
Preparation of reagents	<ol style="list-style-type: none"> 1. Ferric chloride (10%): Take 10 g of ferric chloride in 100 mL volumetric flask and make up the volume with distilled water. 2. Sulphuric acid (80%): Add 80 mL concentrated sulphuric acid into 20 mL distilled water. 3. Hydrochloric acid containing ferric chloride: The reagent is prepared by adding 1 mL of 10% ferric chloride solution in a 500 mL volumetric flask and making up the volume with concentrated hydrochloric acid.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation
Method of analysis	<p>Hehner test:</p> <ol style="list-style-type: none"> 1. Take 5 mL milk sample in a test tube, add 5 mL distilled water and 0.1 mL of 10% FeCl₃ solution. 2. Mix the contents and add 10 mL sulphuric acid (80%) from the side of the test tube. <p>Leech Test:</p> <p>Using milk as a medium:</p> <ol style="list-style-type: none"> 1. Take 5 mL milk in a test tube, add 3 mL hydrochloric acid containing ferric chloride. 2. Heat on direct flame for 1 min and observe for colour. <p>Using whey as a medium:</p> <ol style="list-style-type: none"> 1. Take 5 mL milk in a test tube, add 3 mL hydrochloric acid containing ferric chloride (amount of milk and reagent can be increased proportionately to get sufficient filtrate). 2. Filter the content using Whatman No. 1 filter paper. 3. Take filtrate in a test tube and heat on a direct flame for 1 min. 4. Observe the colour.
Inference (Qualitative analysis)	Hehner test: Violet ring at the junction of two layers indicates presence of formaldehyde. The limit of detection is 0.0005 mL formalin/100 mL milk.

	<p>Leech Test: Appearance of violet colour indicates presence of formaldehyde in milk. The limit of detection is: 0.002 mL formalin/100 mL milk (when test is performed in milk) 0.0005 mL formalin/100 mL milk (when test is performed in whey)</p>
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 39-40. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. Kamps, J. J., Hopkinson, R. J., Schofield, C. J., & Claridge, T. D. (2019). How formaldehyde reacts with amino acids. Communications Chemistry, 2(1), 1-14. 3. Pearson's Composition and Analysis of foods, 9th edition, 1991 page 90. 4. IS 1479 (Part II): 1961 (Reaffirmed Year: 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Hydrogen Peroxide in Milk

Method No.	FSSAI 01.017:2021	Revision No. & Date	0.0
Scope	This method is for the detection of presence of hydrogen peroxide in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Vanadium pentoxide: Should be handled as a carcinogen with extreme caution. It may react violently with chlorine trifluoride, lithium and peroxyformic acid. It is incompatible with strong acids like hydrochloric acid, sulphuric acid, nitric acid. 2. Para-phenylenediamine: Ensure there is adequate ventilation while working with this chemical. Avoid formation of dust and breathing vapours, mist or gas. 3. Potassium iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 4. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 		
Principle	<p>Three different methods have been indicated for detection of presence of hydrogen peroxide. In the first method, appearance of pink or red color after addition of vanadium pentoxide reagent in milk samples indicates the presence of hydrogen peroxide in milk. In the second method, para-phenylenediamine gives blue colour in presence of hydrogen peroxide in milk. In the third method, hydrogen peroxide present in milk sample liberates iodine from potassium iodide which in presence of starch gives blue colour.</p>		
Apparatus/ Instruments	<ol style="list-style-type: none"> 1. 100 mL volumetric flask. 2. Analytical balance (Readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Vanadium pentoxide. 2. Para-phenylenediamine. 3. Potassium iodide. 4. Starch. 5. Concentrated sulphuric acid (Approximately 36.8 N). 		

Preparation of reagents	<ol style="list-style-type: none"> 1. Vanadium pentoxide solution: Dissolve 1 g of vanadium pentoxide (V_2O_5) in 100 mL dilute sulphuric acid (6 mL concentrated sulphuric acid diluted to 100 mL). 2. Para-phenylenediamine solution: Weigh 2.0 g of para-phenylenediamine and dissolve it in distilled water to obtain 100 mL solution i.e. 2% aqueous solution, w/v. Dissolution of para-phenylenediamine in water is difficult and requires thorough mixing. The solution will appear pale yellow. 3. Potassium iodide solution: Weigh 20 g of potassium iodide and dissolve it in distilled water to obtain a 100 mL solution. 4. Starch solution: Take 1 g starch powder and dissolve it in distilled water by heating and make up the volume to 100 mL. 5. Potassium iodide–Starch reagent: Mix equal volumes of 20% potassium iodide solution and 1% starch solution.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Vanadium pentoxide test:</p> <ol style="list-style-type: none"> 1. Add 10 to 20 drops of vanadium pentoxide reagent in 10 mL milk sample and mix. Note the colour of the sample. <p>Para-phenylenediamine test</p> <ol style="list-style-type: none"> 1. Add about 2 mL of milk in a test tube; add equal volume of raw milk. Add two drops of 2% of para-phenylenediamine reagent and mix well. 2. Observe the color of the solution in the tube. <p>Using Potassium iodide and Starch</p> <ol style="list-style-type: none"> 1. Take 1 mL of milk sample in a test tube, add 1 mL of the potassium iodide-starch reagent and mix well. 2. Observe the color of the solution in the tube.
Inference (Qualitative analysis)	<p>Vanadium pentoxide test: Appearance of pink or red colour indicates the presence of hydrogen peroxide in milk.</p> <p>Para-phenylenediamine test: The milk sample adulterated with H_2O_2 develops blue colouration, whereas pure milk samples remain white in colour.</p> <p>Using potassium iodide and starch: The milk sample adulterated with H_2O_2 develops blue colouration, whereas pure milk samples remain white in colour.</p>
Reference	<ol style="list-style-type: none"> 1. AOAC 957.08. 21st Edn. (2019). Official Method- Hydrogen Peroxide in milk. AOAC International, USA. 2. IS 1479 (Part I) - 2016 Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi. 3. Sharma, R.; Rajput, Y.S. & Naik, N.L. (2012). Detection of adulterants in milk – a laboratory manual. NDRI Publication No. 88/2012, NDRI, Karnal, page 10.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Boric acid and Borates in Milk

Method No.	FSSAI 01.018:2021	Revision No. & Date	0.0
Scope	This method is to test the presence of boric acid and borates in milk sample.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. Ammonium hydroxide: Ammonium hydroxide causes skin irritation; contact can lead to severe irritation and burns. If inhaled, mild exposure can cause nose irritation (sneezing, coughing). Exposure to high concentrations must be avoided 2. Caustic soda: Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 		
Principle	Appearance of characteristic red colour on the turmeric paper indicates the presence of boric acid or borax.		
Apparatus/ Instruments	<ol style="list-style-type: none"> 1. 250 mL Erlenmeyer flask. 2. Porcelain dish. 3. Whatman filter paper Grade 2. 4. Analytical balance (Readability 0.01 g). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Turmeric Paper. 2. Concentrated hydrochloric acid (Approximately 11.6 N). 3. Ammonium hydroxide (specific gravity 0.88). 4. Lime water or caustic soda. 5. Ethanol (80%). 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Preparation of Turmeric Paper: Weigh 1.5 to 2.0 g of turmeric powder in 250 mL Erlenmeyer flask and add 100 mL 80% (v/v) ethanol. Shake for 5 min and filter. Collect the filtrate in a flat bottom dish. Dip Whatman filter paper Grade 2 in the clear filtrate. Remove the paper and hang to dry in air. After 1 h, cut the paper into 6 × 1 cm strips and store in tightly stoppered bottle protected from light. 		

Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Method 1:</p> <ol style="list-style-type: none"> 1. Take 20 mL of milk in a porcelain dish and add 1.4 mL of concentrated hydrochloric acid and mix it thoroughly. 2. Dip a strip of turmeric paper in the acidified milk. Appearance of characteristic red colour on the turmeric paper indicates the presence of boric acid or borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$). <p>Method 2.</p> <ol style="list-style-type: none"> 1. Take 25 mL of milk sample in a porcelain dish. Make the sample alkaline with lime water or caustic soda and evaporate it to dryness on water bath. Ignite the dry residue in the dish by heating over low flame until the organic matter is completely charred. 2. Cool the charred residue, re-digest with 15 mL distilled water and add concentrated hydrochloric acid, drop by drop until the ignited residue is dissolved; then add 1 mL of concentrated hydrochloric acid in excess. Immerse a strip of turmeric paper in the solution and dry it in air. 3. Observe the colour change.
Inference (Qualitative analysis)	<ol style="list-style-type: none"> 1. Method 1: Appearance of characteristic red colour on the turmeric paper indicates the presence of boric acid or borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$). The red colour changes to dark blue green on adding ammonium hydroxide, but reappears on re-acidification with hydrochloric acid. 2. Method 2: Appearance of characteristic red colour indicates the presence of boric acid or borax. On addition of ammonium hydroxide, the red turmeric paper turns deep bluish-green in colour. The red colour reappears on re-acidification with hydrochloric acid.
Reference	IS 1479 (Part I): 2016 Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Salicylic acid in Milk

Method No.	FSSAI 01.019:2021	Revision No. & Date	0.0
Scope	This method is to test the presence of salicylic acid in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 3. Ferric chloride: It is a corrosive chemical. Substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. It can react with metals to form flammable and potentially explosive hydrogen gas. 		
Principle	A violet colour outcome by adding ferric chloride solution indicates presence of salicylic acid.		
Apparatus/Instrument	<ol style="list-style-type: none"> 1. Separating funnel. 2. Centrifuge. 3. Analytical balance (Readability 0.01 g). 4. Steam bath 5. Whatman No. 1 filter paper. 		
Materials and reagent	<ol style="list-style-type: none"> 1. Concentrated hydrochloric acid (Approximately 11.6 N). 2. Diethyl Ether. 3. Ferric Chloride. 4. Ammonium hydroxide (specific gravity 0.88). 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Dilute hydrochloric acid: dilute one part of acid with three parts of distilled water) 2. Neutral ferric chloride solution, 0.5% (v/v): Dissolve 0.5 g of ferric chloride in distilled water and make up the volume to 100 mL with distilled water. Add ammonium hydroxide solution dropwise to the prepared solution till brown colour precipitates appear. Filter the solution and use the clear filtrate. 		

Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Take 100 mL of milk in a separating funnel, add 5 mL of dilute hydrochloric. Shake until curdled and filter. 2. Extract the filtrate with 50 to 100 mL diethyl ether and wash the ether layer with two 5 mL portions of water. 3. Evaporate the ether on steam bath and add one drop of ferric chloride solution. 4. Observe the colour change.
Inference (Qualitative analysis)	A violet colour indicates presence of salicylic acid.
Reference	<ol style="list-style-type: none"> 1. A.O.A.C 975.30. 21st Edn. (2019). Official method Salicylic acid in Food and Beverages. AOAC International, USA. 2. IS 1479 (Part II): 1961 (Reaffirmed Year: 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Maltodextrin in Milk

Method No.	FSSAI 01.020:2021	Revision No. & Date	0.0
Scope	This method is for the detection of maltodextrin in milk by Iodine test.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Iodine: It is not compatible with combustibles, strong bases, halogens and ethanol. It reacts violently or explosively with acetylene, acetaldehyde, metal hydrides and metal carbides. 2. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 3. Citric acid: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Excessive intake of citric acid may cause erosion of the teeth. Causes respiratory tract irritation. Repeated exposure may cause sensitization dermatitis. 		
Principle	This test is based on the reaction of Maltodextrin with iodine. Similar to starch, maltodextrin also forms complex with iodine. The dextrans having chain length of 8 DP (degree of polymerization) to 15 DP forms complex with iodine giving yellow-brown to brown to reddish purple colour as chain length increases (Thoma and French, 1960). While, the chain length more than 45 DP gives blue colour.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Conical flask. 3. Water bath. 4. Whatman No. 1 filter paper. 5. Analytical balance (Readability 0.01 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Potassium iodide. 2. Iodine crystals. 3. Citric acid monohydrate. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Iodine solution (1%): Dissolve 2.5 g potassium iodide in 100 mL distilled water, then add 1 g pure iodine crystals. Prepare iodine solution at least a day before as iodine dissolves slowly. 2. Citric acid (5%): Dissolve 5 g citric acid monohydrate in distilled water and make up the volume to 100 mL. 		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.		

Method of analysis	<ol style="list-style-type: none"> 1. Take 20 mL milk in a conical flask, warm the milk to 70-80 °C either on direct flame or boiling water bath. 2. Add 5% citric acid solution drop wise in the milk with gentle stirring until clear coagulation occurs (approximate consumption of citric acid required would be 1.5-2 mL). 3. Flask, if stirred vigorously will result in fine curd particles which may impact colour observation. 4. Filter the contents using Whatman No. 1 filter paper. 5. Take 5 mL filtrate in a test tube, add 0.25 mL of 1% iodine solution. 6. Mix the contents and observe for colour development.
Inference (Qualitative analysis)	Development of red-brown colour indicates adulteration of milk with maltodextrin, whereas pure milk remains yellow in colour. In case sample is preserved with formaldehyde, adulterated milk will show same red brown colour. Limit of detection is 0.1g/ 100 mL milk.
Reference	<ol style="list-style-type: none"> 1. Aparnathi, K.D.; Shaikh, A.I. & Patel, S.I. (2020). Qualitative Tests for Detection of Common Adulterants in Milk. AAU Publication No. RES-1:16:2020:500, Anand, Page 30-31. Published by Director of Research, Anand Agricultural University (AAU), Anand (Gujarat). 2. Thoma, J.A., & French, D. (1960). The Starch-Iodine-Iodide Interaction. Part I. Spectrophotometric Investigations¹. Journal of the American Chemical Society, 82(16), 4144-4147.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Alkaline Phosphatase in Milk

Method No.	FSSAI 01.021:2021	Revision No. & Date	0.0
Scope	This method is for the detection of alkaline phosphatase in milk. It may be noted that the test is applicable to recently pasteurized milk as it has been reported that denatured alkaline phosphatase is activated during storage of pasteurized milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium Carbonate: If improperly handled, or in the event of accidental exposure, sodium carbonate can pose several hazards to people's health and safety. Inhalation of this chemical can lead to adverse effects such as respiratory tract irritation, coughing, shortness of breath, and pulmonary edema. 2. Sodium bicarbonate: It might increase blood pressure. People who already have high blood pressure should avoid sodium bicarbonate. Low potassium levels in the blood: Sodium bicarbonate might lower potassium blood levels. People who already have low levels of potassium should avoid sodium bicarbonate. 3. Disodium para nitrophenyl phosphate: Eye and skin Contact - Rinse immediately with plenty of water for at least 15 min., also under the eyelids. Inhalation- Remove to fresh air. Ingestion - Clean mouth with water and drink afterwards plenty of water. Get medical attention if symptoms occur. 4. Para-nitrophenol: Contact may severely irritate skin and eyes. Poisonous by ingestion and moderately toxic by skin contact. 5. Trichloroacetic acid: It is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Breathing trichloroacetic acid can irritate the nose and throat. Breathing trichloroacetic acid can irritate the lungs causing coughing and/or shortness of breath. 6. Sodium hydroxide: It is very corrosive. It can cause irritation to the eyes, skin, and mucous membrane; an allergic reaction; eye and skin burns; and temporary loss of hair. Workers may be harmed from exposure to sodium hydroxide. The level of harm depends upon the dose, duration, and work being done 		
Principle	This test is a routine quality control test for assessing the efficiency of pasteurization of milk. The principle of this method is that, disodium		

	<p>para-nitrophenyl phosphate which is used as a substrate for alkaline phosphatase, liberates a yellow-coloured compound para-nitrophenol, under the alkaline conditions in the presence of alkaline phosphatase. This liberated yellow colored product is measured spectrophotometrically at 405 nm. <i>p</i>-Nitrophenol when present in the acidic medium remains in an undissociated colorless form, while it readily gets converted into its quinoid structure under alkaline conditions to form a yellow-coloured product.</p>
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube. 2. Pipettes. 3. Volumetric flask. 4. Water bath. 5. Whatman No. 1 filter paper. 6. Funnel. 7. Tripod stand. 8. UV-Visible Spectrophotometer. 9. Analytical balance (Readability 0.01 g).
Materials and Reagents	<ol style="list-style-type: none"> 1. Sodium carbonate. 2. Sodium bicarbonate. 3. Disodium para nitrophenyl phosphate. 4. Para-nitrophenol. 5. Trichloroacetic acid. 6. Sodium hydroxide.
Preparation of reagents	<ol style="list-style-type: none"> 1. Carbonate-bicarbonate buffer (pH 10.0): Dissolve 3.5 g sodium carbonate (Na₂CO₃) and 1.5 g sodium bicarbonate (NaHCO₃) in 1 L of distilled water and adjust the pH to 10. 2. Buffer substrate: Dissolve 0.15 g of disodium <i>p</i>-nitrophenyl phosphate in the carbonate-bicarbonate buffer and make the volume to 100 mL with the buffer. Store the solution at 4 °C and protect from light. This solution is stable for one week. 3. Trichloroacetic acid (TCA): Weigh 28 g of trichloroacetic acid and dissolve in distilled water and make up the volume to 100 mL using distilled water in a volumetric flask. 4. Sodium hydroxide: 14% solution in distilled water. <p>Reagents for Standard Curve Preparation</p> <ol style="list-style-type: none"> 1. Stock <i>p</i>-nitrophenol (PNP) solution: Dissolve 100 mg of PNP in 100 mL distilled water. Working PNP solution: Dissolve 1.0 mL stock solution in 100 mL of distilled water
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.

<p>Method of analysis</p>	<p>Measurement in milk sample</p> <ol style="list-style-type: none"> 1. Take 3.5 mL of carbonate-bicarbonate buffer in a test-tube and add 5 mL buffer substrate, keep the tube in a water bath for 5 min at 37 °C. 2. After the above-mentioned pre-incubation, add 0.5 mL of milk (previously diluted 1:10 with distilled water) and mix the contents. A corresponding blank is carried out using boiled milk (inactivated enzyme) 3. Incubate the tubes for 30 min at 37 °C. 4. Add 1 mL of TCA to the tubes and mix the contents properly. 5. Filter the content of the test tube through filter paper (Whatman no. 1). 6. To 5 mL of filtrate in a test tube, add 0.5 mL of sodium hydroxide. Centrifuge, if necessary. 7. Measure the absorbance of developed color in a spectrophotometer at 405 nm. <p>Preparation of standard curve</p> <ol style="list-style-type: none"> 1. Prepare solution for different concentration (0-50 µg) p-nitrophenol by serially diluting the working solution. 2. Add 0.5 mL of sodium hydroxide. 3. Measure the absorbance of in a spectrophotometer at 405 nm and calculate the enzyme concentration from the standard curve 																								
<p>Calculation with units of expression</p>	<p>The concentration of p-nitrophenol in the sample is calculated using the standard curve.</p> <p>Calculations:</p> <ol style="list-style-type: none"> 1. Preparation of graph of PNP Concentration v/s Absorbance 1 mL stock PNP solution = 1 mg PNP = 1000 µg PNP Dissolve 1.0 mL stock solution = 1.0 mg PNP in 100 mL H₂O 100 mL working PNP solution = 1000 µg PNP 1 mL working PNP solution = 10 µg PNP 0.1 mL working PNP solution = 1.0 µg PNP <p>Observation table for standard curve:</p> <table border="1" data-bbox="540 1661 1458 1896"> <thead> <tr> <th>Test tube</th> <th>Working PNP soln. (mL)</th> <th>H₂O (mL)</th> <th>14% Sodium hydroxide (mL)</th> <th>Conc. PNP (µg)</th> <th>Absorbance at 405 nm</th> </tr> </thead> <tbody> <tr> <td>Blank</td> <td>0.0</td> <td>5.0</td> <td>0.5</td> <td>0.0</td> <td></td> </tr> <tr> <td>1</td> <td>0.5</td> <td>4.5</td> <td>0.5</td> <td>5.0</td> <td></td> </tr> <tr> <td>2</td> <td>1.0</td> <td>4.0</td> <td>0.5</td> <td>10</td> <td></td> </tr> </tbody> </table>	Test tube	Working PNP soln. (mL)	H ₂ O (mL)	14% Sodium hydroxide (mL)	Conc. PNP (µg)	Absorbance at 405 nm	Blank	0.0	5.0	0.5	0.0		1	0.5	4.5	0.5	5.0		2	1.0	4.0	0.5	10	
Test tube	Working PNP soln. (mL)	H ₂ O (mL)	14% Sodium hydroxide (mL)	Conc. PNP (µg)	Absorbance at 405 nm																				
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1	0.5	4.5	0.5	5.0																					
2	1.0	4.0	0.5	10																					

	3	1.5	3.5	0.5	15	
	4	2.0	3.0	0.5	20	
	5	2.5	2.5	0.5	25	
	6	3.0	2.0	0.5	30	
	7	3.5	1.5	0.5	35	
	8	4.0	1.0	0.5	40	
	9	4.5	0.5	0.5	45	
	10	5.0	0.0	0.5	50	
	<p>2. Express the results in terms of enzyme units. One unit of alkaline phosphatase is defined as the preparation liberating 1 µg p-nitrophenol (PNP) in 30 min at 37 °C. The concentration of p-nitrophenol in the sample can be calculated using the standard curve.</p> <p>All the samples giving readings in excess of 10 µg shall be recorded as unsatisfactory.</p>					
Inference (Qualitative analysis)	Pasteurized milk giving readings less than 10 µg of p-nitrophenol shall be recorded as negative for alkaline phosphatase.					
Precautions	The sample of milk should be examined as soon as possible after arrival at the laboratory. If not examined immediately, store between 3 to 5 °C until examined but not for more than 48 hours. The milk sample must be brought to room temperature (25 ± 3 °C) before being tested.					
Reference	<p>1. IS 8479 (Part I): 1977. Method for determination of Phosphatase activity in milk and milk products. Routine method. Bureau of Indian Standards, New Delhi.</p> <p>2. Aschaffenburg, R., & Mullen, J. E. C. (1949). 381. A rapid and simple phosphatase test for milk. Journal of Dairy Research, 16(1), 58-67.</p>					
Approved by	Scientific Panel on Methods of Sampling and Analysis					

Checking Efficiency of Sterilization in Liquid Milk

Method No.	FSSAI 01.022:2021	Revision No. & Date	0.0
Scope	This method is for checking the efficiency of sterilization in liquid milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <p>1. Ammonium sulphate: May explode if mixed with oxidizers, such as potassium nitrate, potassium nitrite, and potassium chlorate. Ensure there is adequate ventilation while working.</p>		
Principle	<p>The turbidity test depends upon the denaturation of proteins of milk, especially albumin after sterilization. When solutions of inorganic salts or acids are added, albumin separates with the casein. The sample after treatment with ammonium sulphate is filtered, and heating of the filtrate shows turbidity due to the presence of albumin on account of insufficient heat treatment. If milk has been sterilized properly, all albumin will have been precipitated and no turbidity will be produced. The test is not suitable for UHT milk.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Conical flask 50mL. 2. Cylinder 25 mL. 3. Test tubes 150 X 16 mm. 4. Funnels, 6 cm diameter. 5. Beaker, 400 mL. 6. Whatman No. 12 or equivalent, 12.5 cm folded filter paper. 7. Analytical balance (Readability 0.01 g). 		
Materials and reagents	Ammonium sulphate.		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Pipette 20 mL of milk in a 50 mL conical flask, add 4.0±0.1 g of ammonium sulphate. 2. Shake the flask till the ammonium sulphate is completely dissolved. 3. Allow the mixture to settle for 5 min; filter through a folded filter paper in a test tube. 4. Keep about 5 mL of the above filtrate in a boiling water bath for 5 min. 5. Cool the tube in a beaker of cold water and examine the contents for turbidity by moving the tube in front of an electric light shaded from the eyes of the observer. 		

Inference (Qualitative analysis)	The milk is considered sterilized when the filtrate shows no turbidity.
Reference	<ol style="list-style-type: none"> 1. IS 4238:2000 Sterilized and Ultra High Temperature Sterilized Milk — Specification. Bureau of Indian Standards, New Delhi. 2. F.A.O Manual of Food Quality Control 14 / 8 page 26.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Fat Content in Milk

Method No.	FSSAI 01.023:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 5. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 6. Amyl alcohol: It is a highly flammable liquid. Keep away from heat/sparks/open flames/hot surfaces. Perform all operations under a fume hood. 		
Principle	<p>Fat content in liquid milk sample can be estimated either by Gerber method (volumetric method) or Rose-Gottlieb method (gravimetric method). The Gerber method is suitable as a routine or screening test. It is an empirical method and reproducible results can be obtained if procedure is followed correctly. The milk is mixed with sulphuric acid and iso-amyl alcohol in a special Gerber tube, permitting dissolution of the protein and release of fat. The tube is centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the fat content of the milk sample. This method can be used for routine purposes.</p>		

	<p>Rose-Gottlieb Method is a gravimetric method in which fat globule membrane of milk fat globules is ruptured by addition of ammonia to milk sample to liberate the fat. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.</p>																						
<p>Apparatus/Instruments</p>	<ol style="list-style-type: none"> 1. Gerber butyrometer, 6, 8 and 10 percent (ISI marked). 2. Pipette: 10.75 ± 0.03 mL for milk at 27°C. 3. Automatic measure or tilt measure for dispensing Gerber sulphuric acid: 10 ± 0.25 mL at 27°C. 4. Automatic measure or tilt measure for dispensing iso-amyl alcohol: 1 ± 0.05 mL at 27°C. 5. Lock stoppers for butyrometer. 6. Lock stopper key. 7. Water-bath: The water-bath shall be made of a suitable material (e.g. stainless steel). It shall be capable of being maintained at $65 \pm 2^\circ\text{C}$ and shall be of sufficient depth as to support the butyrometer in vertical position with their scale completely immersed. The bath shall be fitted with horizontal perforated plates to hold the butyrometer and shall also carry a suitable thermometer. 8. Gerber Centrifuge. The centrifuge may be hand-driven or electric driven. The centrifuge shall be capable of producing within 2 min when fully loaded, a relative centrifugal acceleration of $350 \pm 50 g_n$ at the outer end of the butyrometer stopper. This acceleration is produced by centrifuges with the following effective radius (horizontal distance between the centre of the centrifuge spindle and the outer end of the butyrometer stopper) if operated at the speed indicated against each: <table border="1" data-bbox="613 1371 1386 1890"> <thead> <tr> <th>Effective Radius (mm)</th> <th>Revolution Per Min (± 70 rev/min)</th> </tr> </thead> <tbody> <tr><td>240</td><td>1140</td></tr> <tr><td>245</td><td>1130</td></tr> <tr><td>250</td><td>1120</td></tr> <tr><td>255</td><td>1110</td></tr> <tr><td>260</td><td>1100</td></tr> <tr><td>265</td><td>1090</td></tr> <tr><td>270</td><td>1080</td></tr> <tr><td>275</td><td>1070</td></tr> <tr><td>300</td><td>1020</td></tr> <tr><td>325</td><td>980</td></tr> </tbody> </table> 	Effective Radius (mm)	Revolution Per Min (± 70 rev/min)	240	1140	245	1130	250	1120	255	1110	260	1100	265	1090	270	1080	275	1070	300	1020	325	980
Effective Radius (mm)	Revolution Per Min (± 70 rev/min)																						
240	1140																						
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270	1080																						
275	1070																						
300	1020																						
325	980																						

	<p>Note: The relative centrifugal acceleration (g_n) produced in a centrifuge is given by the following formula:</p> $1.12 \times 10^{-6} r n^2$ <p>where r = effective horizontal radius in mm, and n = speed in revolutions per min</p> <ol style="list-style-type: none"> 9. Mojonnier fat extraction flask or any other suitable extraction tube (as per IS specification). 10. Cork or stopper of synthetic rubber unaffected by usual fat solvents. 11. 100 mL flat bottom flask with G/G joint or stainless steel or aluminium dishes of 5.5 cm height and 9 cm diameter or glass bowl. 12. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C.
Materials and reagents	<ol style="list-style-type: none"> 1. Ammonia solution (Sp. Gr. 0.91). 2. Ethyl alcohol (95%). 3. Diethyl ether, peroxide-free. 4. Petroleum ether, boiling range 40-60 °C. 5. Concentrated Sulphuric acid (Approximately 36.8 N). 6. Iso amyl alcohol (furfural free). It should have density between 0.807 to 0.809 g/mL at 27 °C.
Preparation of Reagents	<ol style="list-style-type: none"> 1. Gerber Sulphuric acid: Take required volume of water in a Pyrex flask (generally 100 mL of water is required for 900 mL of concentrated sulphuric acid) kept in a basin of ice-cold water. Carefully add the commercial sulphuric acid in small quantities at a time keeping the container sufficiently cold and mix gently. After cooling the flasks, check the specific gravity of Gerber acid with hydrometer and if necessary, adjust the Gerber acid to the correct specific gravity with addition of water or acid taking same precautions as before till specific gravity is in the range of 1.807 to 1.812 g/mL at 27 °C (or 1.815 to 1.820 g/mL at 20 °C). Store the prepared acid in a glass stoppered bottle to avoid absorption of water.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Method 1: Gerber Method</p> <ol style="list-style-type: none"> 1. Transfer 10 mL of sulphuric acid into a butyrometer tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. Mix the milk sample gently but thoroughly and fill the milk pipette above the graduation line. 2. Wipe the outside of the pipette and allow the milk level to fall so that the top of meniscus is level with the mark. 3. Run the milk into the butyrometer tube along the side wall without wetting the neck, leave to drain for three sec and touch the pipette's tip once against the base of the neck of the butyrometer tube.

4. Add 1 mL of amyl alcohol, close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid.
5. Keep in water bath at 65 ± 2 °C for at least 3 min and not less than 10 min taking care to have casein particles if any to dissolve fully, and centrifuge for 5 mins at 1100 rpm (approximately 300 g).
6. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge.
7. Allow the centrifuge to come to rest, remove the butyrometer tubes and place in water bath at 65 ± 2 °C for at least 3 min and not less than 10 min.
8. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key.
9. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid.
10. When readings are being taken, hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye, and then read the butyrometer to the nearest half of the smallest scale division.

In homogenised milk, fat separates with more difficulty and centrifuging more than once may be required. Below procedure should be followed for homogenized milk:

1. **In case of homogenized milk**, obtain the second value of fat content by repeating centrifugation. If the second value does not exceed the first value by more than half a smallest scale division of the butyrometer, the second value shall be recorded as the fat content of the milk.
2. If the second value exceeds the first value by more than half a smallest scale division, repeat the centrifugation and obtain a third value for the fat content. If the third value does not exceed the second value by more than half a smallest scale division, the third value shall be recorded as the fat content of the milk.
3. If the third value exceeds the second value by more than half a smallest scale division, repeat the centrifugation and obtain fourth value for the fat content. The fourth value shall be recorded as the fat content of the milk, but if this value exceeds the third value by more than half a smallest scale division, it should be regarded as of doubtful accuracy.
4. If even after the several centrifuging's, the fat is turbid or dark in colour or if there is white or black material at the bottom of the fat column the value for fat content would not be accurate.
5. Holding the tubes too long at 65 °C or above, results in esterification of

the amyl alcohol with a consequent increase in the volume of the fat layer.


6. In case of old samples, if necessary, the concentration of sulphuric acid may be increased from 90-91% to 92-93% to felicitate better dissolution.

Note: The butyrometer must always be emptied without delay and the highly acidic waste disposed of appropriately. The tubes may be cleaned with chromic acid.

Method 2: Rose-Gottlieb Method

1. Weigh accurately about 10 g of sample (liquid milk) in beaker.
2. Add 1.25 mL of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used), mix and shake thoroughly.
3. Transfer the content to extraction tubes.
4. Add 10 mL ethyl alcohol and mix again.
5. Add 25 mL of diethyl ether (peroxide free) stopper and shake vigorously for about a min, then add 25 mL petroleum ether (boiling range 40-60 °C and shake again vigorously for about half a min. Let it stand until the upper ethereal layer has separated completely and is clear (alternatively use low r.p.m. Mojonnier centrifuge).
6. If there is a tendency to form emulsion, a little alcohol may be added to help separation of the layers. Decant off the clear ethereal layer into a suitable vessel (flask, glass bowl, aluminium dish, etc.).
7. Wash the delivery end of the extraction tube with a little ether and add the washings to the flask.
8. Repeat twice extraction of the liquid remaining in the extraction tube using 15 mL of each solvent every time, add the ethereal extract to the same container and evaporate off completely.
9. Dry the flask in an air oven at 102 ± 2 °C for 2 h, cool in a desiccator and weigh.
10. Heat the flask again in the oven for 30 min. Cool in a desiccator and weigh. Repeat the process of heating and cooling and weighing until the difference between two successive weights does not exceed 1 mg.
11. Wash out the fat from the flask with petroleum ether carefully leaving any insoluble residue in the flask.
12. Dry the flask in the oven and reweigh. The difference in weights represents the weight of fat extracted from the milk.
13. Correct weight of extracted fat by blank determination on reagents used. Difference between duplicate determinations obtained simultaneously by the same analyst should not be more than 0.07 g fat

	/ 100g product.
Calculation with units of expression	<p>Method 1: Gerber Method: See the method of analysis.</p> <p>Method 2: Rose-Gottlieb Method:</p> $\text{Fat \% (m/m)} = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Reference	<ol style="list-style-type: none"> 1. F.A.O. Manual of Food Quality Control, 14/8, page 8. 2. IS 1479 (Part II): 1961 (Reaffirmed Year: 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi. 3. AOAC 905.02. 21st Edn. (2019). Official method –Fat in milk. AOAC International, USA. 4. IS 1224: 1977 (Reaffirmed year 2018). Determination of fat by Gerber Method. Part 1 - Milk. Bureau of Indian Standards, New Delhi. 5. ISO 1211: 2010. Milk — Determination of fat content — Gravimetric method (Reference method). International Organization for Standardization, Geneva. 6. Pearson’s Composition and analysis of foods, 9th edn., 1991 page 538.
Approved by	Scientific Panel on Methods of Sampling and Analysis

		Determination of Solids-Not-Fat in Milk	
Method No.	FSSAI 01.024:2021	Revision No. & Date	0.0
Scope	This method is for the determination of Solids-Not-Fat (SNF) in milk samples by gravimetric method.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	<p>In this procedure, first of all total solids in the given sample are estimated. Fat content (estimated by FSSAI 01.023:2021) is subtracted from total solids to calculate at SNF content in the sample.</p> <p>For estimating total solids, a known quantity of milk is dried on a boiling water bath. Subsequently, the sample is dried in hot air oven at 102 ± 2 °C and from the weight of the residue, the total solids content in milk is determined.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical Balance (Readability 0.0001 g). 2. Desiccator provided with an efficient desiccant (for example freshly dried silica gel with a hydrometric indicator). 3. Boiling water bath provided with openings of adjustable size. 4. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 5. Flat bottomed dishes of height 20-25 mm, diameter 50-75 mm and made of appropriate material (stainless steel, nickel or aluminium) provided with well fitted readily removable lids. 6. Water bath capable of being maintained at 35 - 40 °C. 		
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.		
Method of analysis	<p>Determination of Total Solids:</p> <ol style="list-style-type: none"> 1. Transfer sample to a beaker, warm slowly to 35-40 °C on a water bath with careful mixing to incorporate any cream adhering to the sample. Cool the sample quickly to room temperature (25 ± 3 °C). 2. Heat a dish with its lid alongside in the drying oven maintained at 102 ± 2 °C for at least 1 h. Place the lid on the dish and immediately transfer to a desiccator. 3. Allow to cool to room temperature (at least 30 min) and weigh to the nearest 0.1 mg. 4. Add 5 mL of prepared sample, place the lid on the dish and weigh again. 		

	<p>5. Place the dish without the lid on a vigorously boiling water bath in such a way that the bottom of the dish is directly heated by the steam.</p> <p>6. Continue heating till most of the water is removed.</p> <p>7. Remove the dish from the water bath, wipe the underside and place it in the oven alongside the lid and dry it in the oven maintained at 102 ± 2 °C for 2 h; place the lid and transfer to the desiccator.</p> <p>8. Allow the dish to cool and weigh to the nearest 0.1 mg.</p> <p>9. Again, heat the dish with its lid alongside in the oven maintained at 102 ± 2 °C for 1 h. Place the lid on the dish and immediately transfer to the desiccator.</p> <p>10. Allow to cool and weigh again. Repeat the operation again until the difference in the two consecutive weighing does not exceed 1 mg. Record the lowest mass</p> <p>Determination of fat: Refer method for determination of fat in milk (FSSAI 01.023:2021).</p>
<p>Calculation with units of expression</p>	$\text{Total Solids Content (\%)} = \frac{M_2 - M}{M_1 - M} \times 100$ <p>Where,</p> <p>M is the mass in g of empty dish along with lid; M₁ is the initial mass in g of dish with lid and test portion; M₂ is mass in g of dish with lid and dried test portion Round the value obtained to nearest 0.01 % (m/m)</p> <p>SNF Content (%) = Total Solid (%) - Fat (%)</p>
<p>Reference</p>	<p>IS 12333 - 2017/ ISO 6731: 2010. Milk, Cream and Evaporated milk. – Determination of total Solids Content -reference method. Bureau of Indian Standards, News Delhi.</p>
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>

Determination of Total Nitrogen in Milk by Kjeldahl Method

Method No.	FSSAI 01.025:2021	Revision No. & Date	0.0
Scope	This method describes the determination of total nitrogen in milk by Kjeldahl method.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	<p>In this manual, the three commonly used variants of Kjeldahl method have been presented.</p> <p>Method 1. Macro Kjeldahl Method by ISO procedure (ISO 8968-1:2014)</p> <p>Method 2. Block Digestion/ Steam Distillation method (ISO 8968-2:2001)</p> <p>Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)]</p>		

	<p>Any of the above method can be employed in the laboratory depending on the availability of the equipment in the laboratory.</p> <p>The method involves three major steps. In the first step, the protein is digested using concentrated sulphuric acid in presence of a catalyst (potassium sulphate/copper sulphate). In this step, all the organic material is oxidized except nitrogen, which is converted to ammonium sulphate (Eq 1). In the second step, the digest is neutralized with alkali to liberate ammonia (Eq 2). The ammonia distilled is collected in boric acid. In the third step, the collected ammonia in boric acid is titrated with standard hydrochloric acid (Eq 3 & 4) in the presence of a methyl red-bromocresol green indicator until the green distillate changes from colourless to pink (methyl red, methylene blue indicator can also be used).</p> <p>Reaction</p> $\text{Protein} \xrightarrow{K_2SO_4, CuSO_4, H_2SO_4} (NH_4)_2SO_4 \quad \text{Eq 1}$ $(NH_4)_2SO_4 + 2NaOH \xrightarrow{\text{Heat}} 2NH_3 + Na_2SO_4 + 2H_2O \quad \text{Eq 2}$ $NH_3 + H_3BO_3 \longrightarrow NH_4^+ \cdot H_2BO_4^- \quad \text{Eq 3}$ $NH_4^+ \cdot H_2BO_4^- + HCl \xrightarrow{\text{(Green)}} NH_4Cl + H_3BO_3 \quad \text{Eq 4}$ <p style="text-align: center;">(Pink at pH < 4.8)</p> <p>The quantity of acid required for titration is equivalent to the concentration of ammonia in the distillate and to the nitrogen content of the original protein containing sample. The total amount of protein is calculated using the conversion factor of 6.38.</p> <p>The basic principle of the Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)] is also same as indicated above. However, in this variant of the method, liberated ammonia is quantitatively distilled into a measured volume of standard hydrochloric acid or sulphuric acid.</p>
<p>Apparatus/Instruments</p>	<p>Method 1. Macro Kjeldahl Method by ISO procedure (ISO 8968-1:2014)</p> <ol style="list-style-type: none"> 1. Kjeldahl flasks: Kjeldahl, hard, moderately thick, well-annealed glass, 500 or 800 mL capacity.

2. Distillation flask: Same Kjeldahl flask, fitted with rubber stopper through which lower end of sufficient rubber bulb or trap passes to prevent mechanical carryover of sodium hydroxide during distillation. Connect upper end of the bulb to condenser tube by rubber tubing. Use graduated 500 mL Erlenmeyer titration flask to collect distillate. Trap outlet of condenser in manner to ensure complete absorption of ammonia distilled into boric acid solution.
3. Digestion apparatus, to hold the Kjeldahl flasks in an inclined position approximately 45 °C along with electric heater or gas burners that do not heat the flasks above the level of their contents, and with a fume extraction system. The heater source should be adjustable to determine the maximum heater setting to be used during digestion. Preheat the heat source at the heater setting for evaluation. In the case of a gas heater, the preheat period shall be 10 min and for an electric heater the preheat period shall be 30 min. Determine the heater setting that brings 250 mL of water (including 5-10 boiling aids) with an initial temperature of 25 °C to a rolling boil in 5-6 min for each type of heaters. This is the maximum heater setting to be used during digestion.
4. Conical or Erlenmeyer flask: 500 mL capacity, graduated at every 200 mL.
5. Burette: 50 mL capacity, graduated at least at every 0.1 mL.
6. Boiling aid, having a mesh size 10. Use plain, high purity amphoteric alundum granules. Do not reuse the aids. Glass beads of approximately 5 mm diameter are also used, but they do not promote as efficient boiling as the alundum granules and more foaming problems will be encountered during digestion with glass beads.
7. Measuring cylinders: 50, 100 and 500 mL capacities, graduated.

Method 2. Block Digestion/ Steam Distillation method (ISO 8968-2:2001)

1. Digestion block: Aluminium alloy block or equivalent apparatus, fitted with an adjustable temperature control device for measuring block temperature.
2. Digestion tubes: 250 mL capacity, suitable for use with digestion flask.
3. Exhaust manifold: Suitable for use with the digestion tubes.
4. Centrifugal scrubber apparatus or filter pump or aspirator: Constructed of acid resistant material and for use with main water supply.
5. Measuring cylinder: 25, 50 and 100 mL capacities, graduated.

6. Distillation unit: Manual /semi-automatic/automatic, for steam distillation, suited to accept the 250 mL digestion tubes and 500 mL conical flasks.
7. Conical or Erlenmeyer flask: 500 mL capacity, graduated at 200 mL.
8. Burette: 50 mL capacity, graduated at least at every 0.1 mL.

Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)]

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

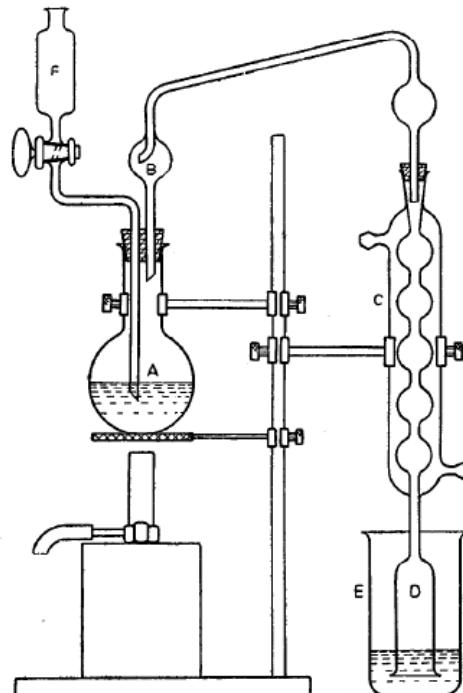


Fig 1: Distillation assembly (IS 7219:1973)

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

Materials and Reagents

Use all the reagents of AR Grade quality, unless otherwise specified, and use distilled or demineralized water only.

	<p>For Method 1. Macro Kjeldahl Method by ISO procedure (ISO 8968-1:2014) and Method 2. Block Digestion/ Steam Distillation method (ISO 8968-2:2001)</p> <ol style="list-style-type: none"> 1. Potassium sulfate (K_2SO_4): Nitrogen free or low in nitrogen content. 2. Copper (II) sulfate solution 3. Concentrated sulphuric acid: (Approximately 36.8 N) nitrogen free 4. Sodium hydroxide solution, 50%, m/m (low in nitrogen)) 5. Methyl red 6. Bromocresol green 7. Ethanol 8. Boric acid solution (H_3BO_3) 9. Standard hydrochloric acid solution: 0.1 ± 0.0005 N. 10. Ammonium sulfate $(NH_4)_2SO_4$ 11. Tryptophan ($C_{11}H_{12}N_2O_2$) or Lysine hydrochloride ($C_6H_{15}ClN_2O_2$): Minimum assay 99%, do not dry these reagents in an oven before use. 12. Sucrose with a nitrogen content of not more than 0.002% (m/m). Do not dry in an oven before use. <p>Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)]</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid (Approximately 36.8 N), nitrogen-free. 2. Mercuric oxide or Metallic Mercury or Copper (II) Sulphate Pentahydrate - nitrogen-free. 3. Potassium sulphate or anhydrous sodium sulphate - nitrogen-free. 4. Zinc Granules 5. Sulphide or thiosulphate Solution - Dissolve 40 g potassium sulphide or 80 g hydrated sodium thiosulphate in 1 L distilled water. 6. Sodium hydroxide -pellets, flakes or solution; nitrogen free. 7. Hydrochloric or sulphuric acid, standard Solution - 0.1 or 0.5 N. Standardize against primary standard and against sodium hydroxide standard solution 8. Sodium hydroxide standard Solution - 0.1 N. Standardize against primary standard (potassium hydrogen phthalate) and against standard acid solution. 9. Methyl Red Indicator
Preparation of reagents	<p>For Method 1. Macro Kjeldahl Method by ISO procedure (ISO 8968-1:2014) and Method 2. Block Digestion/ Steam Distillation method (ISO 8968-2:2001)</p>

	<ol style="list-style-type: none"> 1. Copper (II) sulfate solution: Dissolve 5.0 g of copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water and make up the final volume to 100 mL in a 100 mL volumetric flask. 2. Sodium hydroxide solution, 50%, m/m (low in nitrogen): Dissolve 50 g sodium hydroxide pellets in water and finally make to 100 mL. 3. Indicator solution: Dissolve 0.1 g of methyl red in 95% (v/v) ethanol and dilute to 50 mL with ethanol. Dissolve 0.5 g of bromocresol green in 95% (v/v) ethanol and dilute to 250 mL with ethanol. Mix 1 part of methyl red solution with 5 parts of bromocresol green solution or combine all of both solutions. 4. Boric acid solution (H_3BO_3): Dissolve 40 g of boric acid in hot water, allow the solution to cool and dilute to 1 L. Add 3 mL of methyl red-bromocresol indicator solution, mix and store the solution in borosilicate glass bottle. The solution will be light orange in colour. Protect the solution from light and sources of ammonia fume during storage. 5. Standard hydrochloric acid solution: 0.1 ± 0.0005 N. 6. Ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$]: Minimum assay 99.9% on dried material. Immediately before use dry the ammonium sulfate at 102 ± 2 °C for not less than 2 h. Cool to room temperature (25 ± 3 °C) in a desiccator. 7. Tryptophan or lysine hydrochloride: Minimum assay 99%, do not dry these reagents in an oven before use. 8. Sucrose with a nitrogen content of not more than 0.002% (m/m). Do not dry in an oven before use. <p>Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)]</p> <ol style="list-style-type: none"> 1. Sulphide or Thiosulphate Solution - Dissolve 40 g potassium sulphide or 80 g hydrated sodium thiosulphate in 1 L distilled water. 2. Sodium hydroxide solution; nitrogen free. For solution, dissolve about 450 g solid sodium hydroxide in distilled water, cool, and dilute to 1 L. The specific gravity should be at least 1.36 at 20 °C. 3. Hydrochloric or sulphuric Acid, Standard Solution - 0.1 or 0.5 N. standardize against sodium hydroxide standard solution 4. Sodium hydroxide standard solution - 0.1 N. standardize against primary standard (potassium hydrogen phthalate) and used to standardise acid solution. 5. Methyl Red Indicator - Dissolve 1 g methyl red in 200 mL alcohol.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.

<p>Method of analysis</p>	<p>Method 1. Macro Kjeldahl Method by ISO procedure (ISO 8968-1:2014)</p> <p>Test Portion and Pre-treatment:</p> <ol style="list-style-type: none"> 1. Add to the clean and dry Kjeldahl flask, 5-10 boiling aids, 15 g K₂SO₄, 1.0 mL of the copper sulfate solution, approximately 5 ± 0.1 g of prepared milk sample (or milk product sample containing equivalent amount of protein), weighed to the nearest 0.1 mg, and add 25 mL of concentrated sulphuric acid. 2. Use the 25 mL acid to also wash down any copper sulfate solution, K₂SO₄ or milk left on the neck of the flask. Gently mix the contents of the Kjeldahl flask. <p>Digestion:</p> <ol style="list-style-type: none"> 1. Turn on the fume extraction system of the digestion apparatus prior to beginning the digestion. 2. Heat the Kjeldahl flask and its contents on the digestion apparatus using a heater setting low enough such that charred digest does not foam up the neck of the Kjeldahl flask. 3. Digest at this heat-setting for at least 20 min or until white fumes appear in the flask. Increase the heater setting to half way to the maximum setting as determined previously (See Digestion apparatus) and continue the heating period for 15 min. At the end of 15 min period, increase the heat to maximum setting. 4. After the digest clears (clear with light blue-green colour), continue boiling for 1 h to 1.5 h at maximum setting. The total digestion time will be between 1.8-2.25 h. <p>Note: To determine the specific boiling time required for analysis conditions in a particular laboratory using a particular set of apparatus, for milk analysis, select a high-protein, high-fat milk sample and determine its protein content using different boil times (1 h-1.5 h) after clearing. The mean protein result increases with increasing boil time, becomes consistent and then decreases when boil time is too long. Select the boil time that yields the maximum protein result.</p> <ol style="list-style-type: none"> 1. At the end of digestion, the digest shall be clear and free of undigested material. Allow the acid digest to cool to room temperature (25 ± 3 °C) over a period of approximately 25 min. 2. If the flasks are left on hot burners to cool, it will take longer to reach room temperature (25 ± 3 °C). The cooled digest should be liquid or
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liquid with a few small crystals at the bottom of the flask at the end of 25 min cooling period.

3. Do not leave the undiluted digest in the flask overnight. The undiluted digest may crystallize during this period and it will be very difficult to get that back into the solution to avoid this situation.

Note: Excessive crystallization after 25 min is the result of undue acid loss during digestion and can result in low test values. Undue acid loss is caused by excessive fume aspiration or an excessively long digestion time caused by an incorrect maximum burner setting.

1. After the digest is cooled to room temperature (25 ± 3 °C), add 300 mL of water to 500 mL Kjeldahl flask or 400 mL of water when using 800 mL Kjeldahl flask. Use the water to wash down the neck of the flask too.
2. Mix the contents thoroughly ensuring that any crystals which separate out are dissolved. Add 5-10 boiling aids.
3. Allow the mixture to cool again to room temperature (25 ± 3 °C) prior to the distillation. Diluted digests may be stoppered and held for distillation at a later time.

Distillation:

1. Turn on the condenser water for the distillation apparatus.
2. Add 75 mL of 50% (m/m) sodium hydroxide solution to the diluted digest by carefully pouring the solution down the inclined neck of the Kjeldahl flask, so as to form a clear layer at the bottom of the bulb of the flask. There should be a clean interface between the two solutions.
3. Immediately after the addition of sodium hydroxide solution to the Kjeldahl flask, connect it to the distillation apparatus, the tip of whose condenser outlet tube is immersed in 50 mL of boric acid solution with indicator contained in a 500 mL Erlenmeyer flask.
4. Vigorously swirl the Kjeldahl flask to mix its contents thoroughly until no separate layers of solution are visible in the flask any more. Set the flask down on the burner.
5. Turn on the burner to a setting high enough to boil the mixture; continue distillation until irregular boiling (bumping) starts and then immediately disconnect the Kjeldahl flask and turn off the burner. Turn off the condenser water.
6. The distillation rate shall be such that approximately 150 mL distillate is collected when irregular boiling (bumping) starts and the volume of the contents of the conical flask will be approximately 200 mL.

7. If the volume of distillate collected is less than 150 mL, then it is likely that less than 300 mL of water is added to dilute the digest. The efficiency of the condenser shall be such that the temperature of the contents of conical flask does not exceed 35 °C during distillation.

Titration:

1. Titrate the boric acid receiving solution with standard hydrochloric acid solution (0.1 N) to the first trace of pink colour.
2. Take the burette reading to at least the nearest 0.05 mL. A lighted stir plate may aid visualization of the end point.

Blank Test: Simultaneously carry out a blank test by following the procedure as described above taking all the reagents and replacing the milk sample with 5 mL water and about 0.85 g of sucrose.

Note:

1. The purpose of sucrose in a blank or a recovery standard is to act as organic material to consume an amount of sulphuric acid during digestion that is roughly equivalent to a test portion. If the amount of residual free sulphuric acid at the end of digestion is too low, the recovery of nitrogen by both recovery tests (See Section. Nitrogen recovery test) will be low. If the amount of residual acid present at the end of the digestion is sufficient to retain all the nitrogen, but the temperature and time conditions during digestion were not sufficient to release all the nitrogen from a sample, then the nitrogen recovery will be acceptable.
2. The amount of titrant used in the blank should always be greater than 0.00 mL. Blanks within the same laboratory should be consistent across time. If the blank is already pink before the beginning of titration, something is wrong. Usually, in such cases, the conical flasks are not clean or water from the air that may condense on the outside of the condenser apparatus has dripped down into the collection flask to cause the contamination.

Nitrogen Recovery Test

1. The accuracy of the procedure should be checked regularly by means of following recovery tests, carried out in accordance with procedure as in the preceding steps
2. Check that no loss of nitrogen occurs by using a test portion of 0.12 g of ammonium sulfate along with 0.85 g of sucrose. Add all other reagents (except milk sample) as stated in Step A. Digest and distill under same conditions as for a milk sample

3. The percentage of nitrogen recovered shall be between 99.0 and 100.0% for the given apparatus.
4. In the case recoveries of nitrogen exceed 100%, ammonium sulfate is only useful to determine whether nitrogen loss has occurred or the normality of titrant is lower than the stated value. For recoveries less than 99%, the loss could be in the digestion or distillation step.
5. It is possible to use a mixture of ammonium sulfate and small amount of sulphuric acid (the amount of residual remaining at the end of digestion) in a Kjeldahl flask. Dilute it with the normal value of water, add the normal amount of sodium hydroxide solution and distill.
6. If the nitrogen recovery is still low by the same amount, the loss of nitrogen is in the distillation apparatus and not in that of the digestion. The probable cause might be a leaky tubing in a traditional system or the tips of the condensers not submerged under the surface of boric acid solution early in the distillation. The apparatus should pass this test before going on to check recoveries by the procedure described below.
7. Check the efficiency of digestion procedure by using 0.16 g of lysine hydrochloride or 0.18 g of tryptophan along with 0.67 g of sucrose. Add all other reagents (except milk sample). Digest and distill under same conditions as for a milk sample. At least 98% of the nitrogen shall be recovered. If the recovery is lower than 98% after having a 99 - 100% recovery on ammonium sulfate, then the temperature or time of digestion is insufficient or there is undigested sample material (i.e., char) on the inside of the Kjeldahl flask.
8. The final evaluation of performance is best done by participation in a proficiency testing system, where within and between laboratories statistical parameters are computed based on analysis of milk samples.
9. Lower results in either of the recovery tests (or higher than 100% in case of ammonium sulfate) will indicate failures in the procedure and/or inaccurate concentration of the standard hydrochloric acid solution.

Method 2. Block Digestion/ Steam Distillation method (SO 8968-2:2001)

Test Portion and Pre-treatment:

1. Add to the clean and dry digestion tube, 12 g potassium sulphate, 1.0 mL of the copper sulfate solution, approximately 5 ± 0.1 g of prepared milk sample, weighed to the nearest 0.1 mg, and add 20 mL of concentrated sulphuric acid.

2. Use the sulphuric acid also to wash down and copper sulfate solution, K_2SO_4 or milk left on the upper walls of the digestion tube. Gently mix the contents of the tube.

Note: The amount of acid used in the block digestors is less than that used in the Macro Kjeldahl method because the volumes of acid greater than 20 mL in the block digestion systems gives excessive foaming problem during digestion and variable results. Users of block digestors must note that maintaining sufficient residual sulphuric acid at the end of digestion needs more attention by the analyst in the block digestors than in Macro Kjeldahl method. Excessive acid loss due to over aspiration of fumes is more of concern in block digestors than Macro Kjeldahl method.

Digestion

1. Set the digestion block at a low initial temperature so as to control foaming (approximately a temperature between 180-230 °C). Transfer the tube to the digestion block and place the exhaust manifold which is itself connected to a centrifugal scrubber of similar device in the top of the tube.
2. The suction rate of the centrifugal scrubber or similar device shall be just sufficient to remove fumes. The complete apparatus may need to be kept inside a fume hood.
3. Digest the test portion for 30 min or until white fumes develop, then increase the temperature of digestion block to a temperature between 410-430 °C and continue digestion of the test portion until the digest is clear.

Note: It may be necessary to increase the temperature gradually over a period of approximately 20 min to control foaming. In any event, do not let foam rise higher than 4-5 cm below the surface of exhaust manifold inserted into the top of the digestion tube.

1. After the digest clears (clear with light blue-green colour) continue digestion at a temperature of between 410-430 °C for at least 1 h.
2. During this period the sulphuric acid must be boiling. If visible boiling of the clear liquid is not apparent as bubbles forming at the surface of the hot liquid around the perimeter of the tube, then the temperature of the block may be too low. The total digestion time will be between 1.75-2.5 h.
3. To determine the specific boiling time required for analysis conditions in a particular laboratory using a particular set of apparatus, select a

high-protein, high-fat milk sample and determine its protein content using different boil times (1-1.5 h) after clearing.

4. The mean protein content increases with increase in boil time, becomes consistent and then decreases when boil time is too long. Select the boil time that yields the maximum protein results.
5. At the end of digestion, the digest should be clear and free of undigested material. Remove the tube from the block with the exhaust manifold in place.
6. Allow to cool to room temperature (25 ± 3 °C) over a period of approximately 25 min. The cooled digest should be liquid or liquid with a few small crystals at the bottom of the tube.
7. Do not leave the undiluted digest in the tube overnight. The undiluted digest may solidify and it will be very difficult to get that back into the solution with water.

Note: Excessive crystallization after 25 min is the result of undue acid loss during digestion and can result in low test values. Undue acid loss is caused by excessive fume aspiration or an excessively long digestion time caused by digestion for too long a period at a temperature below the maximum temperature of the analysis. To reduce acid loss, reduce the rate of fume aspiration.

1. After the digest is cooled to room temperature (25 ± 3 °C), remove the exhaust manifold and carefully add 85 mL of water to each tube.
2. Swirl to mix while ensuring that any crystals which separate out are dissolved.
3. Allow the contents of the tube to cool again to room temperature (25 ± 3 °C).

Distillation

1. Turn on the condenser water for the distillation apparatus.
2. Attach the digestion tube containing the diluted digest to the distillation unit.
3. Place a conical flask containing 50 mL of the boric acid solution under the outlet of the condenser, in such a way that the delivery tube is below the surface of the boric acid solution.
4. Adjust the distillation unit to dispense 55 mL of sodium hydroxide solution.

Note: Where 40% (m/m) sodium hydroxide solution is used; the dispensed volume should be adjusted to 65 mL. If the automatic delivery of sodium hydroxide solution is extremely variable due to the partial plugging of the

delivery tubing for the sodium hydroxide, then large variability in duplicate results will occur.

1. As per the manufacturer's instructions, operate the distillation unit in such a way as to steam distill the ammonia liberated by addition of sodium hydroxide solution, collecting the distillate in the boric acid solution.
2. Continue with the distillation process until at least 150 mL of distillate has been collected. Remove the conical flask from the distillation unit.

Titration

1. Titrate the boric acid receiving solution with standard hydrochloric acid solution (0.1 N) to the first trace of pink colour.
2. Take the burette reading to at least the nearest 0.05 mL. A lighted stir plate may aid visualization of the end point.

Blank Test

Simultaneously carry out a blank test by following the procedure as described above taking all the reagents and replacing the milk sample with 5 mL water and about 0.85 g of sucrose. Please see the Note mentioned in Method 1.

Nitrogen Recovery Test - Please see the details mentioned in Method 1.

Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)]

Digestion

1. Accurately weigh 0.7 to 2.2 g of the sample into the digestion flask.
2. Add 0.7 g mercury oxide or 0.65 g mercury or 0.45 g of copper (II) sulphate pentahydrate and 15 g powdered potassium sulphate or anhydrous sodium sulphate, and 25 mL sulphuric acid.
3. Ratio of salt to acid (m/v) should be approximately 1: 1 at the end of digestion for proper temperature control.
4. Digestion may be incomplete at a lower ratio and nitrogen may be lost at a higher ratio.
5. Each gram of fat consumes 10 mL and each gram of carbohydrate 4 mL sulphuric acid during digestion.
6. Place the flask in an inclined position on a heater and heat gently until foaming ceases.
7. A small amount of paraffin or silicon antifoam may be added to reduce foaming. Boil vigorously until the solution becomes clear and then continue boiling it for 1 to 2 h.

	<p>Distillation –</p> <ol style="list-style-type: none"> 1. Cool, add about 200 mL distilled water, and in order to avoid complex formation, add 25 mL of the sulphide or thiosulphate solution when mercury or mercuric oxide is used as catalyst. Mix to precipitate the mercury. 2. Add a few zinc granules to prevent bumping, incline flask, and add without agitation 25 g of sodium hydroxide as solid or equivalent as solution, to make solution strongly alkaline (the thiosulphate or sulphide solution may be mixed with the sodium hydroxide solution before addition to the flask). 3. Immediately connect flask to distillation bulb or trap on condenser, and, with tip of the condenser immersed in a measured quantity standard hydrochloric acid (usually 50 mL, 0.5 N or an appropriate quantity of 0.1 N) in the receiver, rotate flask to mix the contents thoroughly; then heat immediately until all ammonia has distilled over (at least 150 mL distillate). 4. Lower the receiver before stopping distillation and wash tip of condenser with distilled water. Back-titrate excess acid with standard 0.1 N sodium hydroxide, using methyl red as indicator. Correct for blank determination in reagents. <p>Blank Conduct determinations using all reagents and 2 g of sugar.</p>
<p>Calculation with units of expression</p>	<p>For Method 1. Macro Kjeldahl Method by ISO procedure (ISO 8968-1:2014) and Method 2. Block Digestion/ Steam Distillation method (ISO 8968-2:2001)</p> <p>Calculate the nitrogen content, expressed as a percentage by mass, by the following formula:</p> $W_n = \frac{1.4007 \times (V_s - V_B) \times N}{W}$ <p>W_n = nitrogen content of sample, expressed as a percentage by mass; V_s = volume in mL of the standard hydrochloric acid used for sample; V_B = volume in mL of the standard hydrochloric acid used for blank test; N = Normality of the standard hydrochloric acid expressed to four decimal places; W = mass of test portion in g, expressed to nearest 0.1 mg.</p> <p>Express the nitrogen content to four decimal places.</p>

	<p>The crude protein content, expressed as percentage by mass is obtained by multiplying the nitrogen content by 6.38. Express the crude protein results to three decimal places.</p> <p>Method 3. Macro Kjeldahl Method by BIS procedure [IS 7219: 1973 (RA 2015)]</p> <p>Nitrogen content (N) in g= (a - 0.2b) - (c - 0.2d) x 0.007</p> <p>Where, a = volume in mL 0.5 N acid measured for main distillation, b = volume in mL 0.1 N alkali used for back-titrating a, c = volume in mL 0.5 N acid measured for blank distillation, and d = volume in mL 0.1 N alkali used for back-titrating c.</p> <p>OR</p> <p>Nitrogen content (N) in g= (A - B) - (C - D) x 0.0014</p> <p>Where, A = volume in mL 0.1 N acid measured for main distillation, B = volume in mL 0.1 N alkali used for back-titrating A, C = volume in mL 0.1 N acid measured for blank distillation, and D = volume in mL 0.1 N alkali used for back-titrating C.</p> <p>Calculation of Total Protein</p> <p>Protein, percent by mass = $\frac{N \times 100 \times 6.38}{W}$</p> <p>Where, N = mass of nitrogen content in g of original sample, W = mass of sample in g.</p>
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of True Protein in Milk

Method No.	FSSAI 01.026:2021	Revision No. & Date	0.0
Scope	This method describes the determination of true protein in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Trichloroacetic acid: Trichloroacetic acid is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Trichloroacetic acid can irritate the nose and throat. Trichloroacetic acid can irritate the lungs causing coughing and/or shortness of breath. 		
Principle	<p>The method is used for analysis of true protein content by estimating the nitrogen content obtained only from proteins and not from non-protein nitrogen (NPN). In this method, the protein in milk sample is precipitated by the addition of TCA such that the final concentration of TCA in the mixture is approximately 12%. The precipitated proteins are filtered and the filtrate will contain the NPN contents. The nitrogen content of the precipitated protein is then determined by Kjeldahl method which represents true protein nitrogen content of milk</p>		
Apparatus/Instruments	<p>Apart from the apparatus listed in Kjeldahl method for the determination of nitrogen content in milk (Refer method FSSAI 01.025:2021), the following apparatus required are as below:</p> <ol style="list-style-type: none"> 1. Conical flask: 25 mL capacity. 2. Pipettes: 10, 20 mL capacities. 3. Filter funnel: 75 mm diameter. 4. Whatman filter paper: Grade 1 (nitrogen free). 5. Beaker: 50 mL capacity. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Trichloroacetic acid (TCA) 2. Other reagents as already described in Kjeldahl method for the determination of total nitrogen content in milk. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Trichloroacetic acid (TCA) solution (15%, w/v): Dissolve 15.0 g of trichloroacetic acid (CCl₃COOH) in water and dilute to 100 mL in a volumetric flask. <p>Note: Do not use other concentrations of TCA and volumes of solutions than those specified in this procedure. Other concentrations or volumes of solutions will change the performance of the method with respect to its mean value and performance characteristics.</p>		

	Other reagents as already described in Kjeldahl method for the determination of total nitrogen content in milk.
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Test Portion</p> <ol style="list-style-type: none"> 1. Weigh approximately 5 ± 0.1 g, to the nearest 0.1 mg, of the prepared milk sample into a Kjeldahl flask. 2. Immediately add 5.0 ± 0.1 mL of water to the Kjeldahl flask, rinsing any milk on the neck of the flask to the bottom of it. <p>Precipitation and Filtration</p> <ol style="list-style-type: none"> 1. Add 40 ± 0.5 mL of TCA solution to the flask and swirl to mix the contents. Let the flask stand for approximately 5 min to allow the precipitate to settle. 2. Pour the mixture from the Kjeldahl flask through a Whatman filter paper Grade 1 and collect the entire filtrate in a clean, dry conical flask. Some of the precipitate will remain in the Kjeldahl flask and some will be collected on the filter paper. It is not necessary to remove all of the precipitate from the flask. 3. Immediately after pouring the mixture (and so as not to allow any precipitate to dry on the neck of Kjeldahl flask), add, by means of a pipette, 10 mL of TCA solution. 4. Use the acid solution to also rinse any precipitate from the neck of the flask down on to the bottom, swirl to mix the contents. 5. Pour the mixture from the flask through the same filter paper, adding the filtrate to that collected previously. 6. Immediately rinse the neck of the flask with a further 10 mL of TCA solution again. 7. Swirl to mix the contents and again pour the mixture from the flask through the same filter paper, adding the filtrate to that collected previously. This filtrate shall be clear and free of particulate matter. <p>Note: At this point, the filtrate is no longer needed and may be discarded in an appropriate manner.</p> <ol style="list-style-type: none"> 8. Wearing gloves, carefully remove the filter paper from the filter funnel and fold the filter paper to enclose the precipitate. 9. If any precipitate remains on either the inner or outer lip of the Kjeldahl flask, wipe with the folded filter paper so that any precipitate adheres to the paper and then drop the filter paper into the Kjeldahl flask. <p>Digestion and Distillation</p> <ol style="list-style-type: none"> 1. Add the appropriate amount of boiling aids, K_2SO_4, copper (II) sulfate solution and concentrated sulphuric acid as specified in the Macro Kjeldahl method for the determination of total nitrogen content in milk.

	<p>2. Determine the nitrogen content of the precipitate and filter paper by the Macro Kjeldahl method.</p> <p>Titration</p> <ol style="list-style-type: none"> 1. Titrate the boric acid receiving solution with standard hydrochloric acid solution (0.1 N) to the first trace of pink colour. 2. Take the burette reading to at least the nearest 0.05 mL. A lighted stir plate may aid visualization of the end point. <p>Blank Test</p> <ol style="list-style-type: none"> 1. Carry out a blank test as described above. Replace the test portion by a filter paper washed with TCA solution and record the blank value.
Calculation with units of expression	<p>The true protein nitrogen content, expressed as percentage by mass is calculated by the following formula:</p> $W_{PN} = \frac{1.4007 \times (V_S - V_B) \times N}{W_t}$ <p>Where,</p> <p>W_{PN} = true protein nitrogen content of the sample, expressed as a percentage by mass;</p> <p>V_S = volume in mL of the standard hydrochloric acid used for sample;</p> <p>V_B = volume in mL of the standard hydrochloric acid used for blank test;</p> <p>N = Normality of the standard hydrochloric acid expressed to four decimal places;</p> <p>W_t = mass of the test portion in g, expressed to nearest 0.1 mg.</p> <p>Express the results to four decimal places.</p> <p>The true protein content is obtained by multiplying the true protein nitrogen content by 6.38. Express the test result to three decimal places.</p>
Reference	ISO 8968-5/IDF 020-5:2001 - Milk - Determination of nitrogen content - Part 5: Determination of protein-nitrogen content. International Organization for Standardization, Geneva, Switzerland.
Approved by	Scientific Panel on Methods of Sampling and Analysis


Determination of Non-Protein Nitrogen (NPN) in Milk

Method No.	FSSAI 01.027:2021	Revision No. & Date	0.0
Scope	This method describes the determination of non-protein nitrogen in milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Trichloroacetic acid: Trichloroacetic acid is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Trichloroacetic acid can irritate the nose and throat. Trichloroacetic acid can irritate the lungs causing coughing and/or shortness of breath. 		
Principle	In this method, proteins are precipitated from milk by the addition of trichloroacetic acid solution. Final concentration of trichloroacetic acid in the mixture is about 12%. Precipitated milk proteins are removed by filtration. Filtrate contains NPN components of milk. Nitrogen content of filtrate is determined using Kjeldahl method.		
Apparatus/Instruments	<p>Apart from the apparatus listed in Macro Kjeldahl method for the determination of nitrogen content in milk (Refer method FSSAI 01.025:2021), the following apparatus required are as below.</p> <ol style="list-style-type: none"> 1. Conical flask: 25 mL capacity. 2. Pipettes: 10, 20 mL capacities. 3. Filter funnel: 75 mm diameter. 4. Whatman filter paper: Grade 1 (nitrogen free). 5. Beaker: 50 mL capacity. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Trichloroacetic acid (TCA) solution 2. Concentrated Hydrochloric acid (Approximately 11.6 N) 3. Other reagents as already described in Macro Kjeldahl method for the determination of total nitrogen content in milk. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Trichloroacetic acid (TCA) solution (15%, w/v, aq): Dissolve 15.0 g of trichloroacetic acid (CCl₃COOH) in water and dilute to 100 mL in a volumetric flask. 		

	<p>Note: Do not use other concentrations of TCA and volumes of solutions than those specified in this procedure. Other concentrations or volumes of solutions will change the performance of the method with respect to its mean value and performance characteristics.</p> <p>2. Standard hydrochloric acid solution: 0.01 ± 0.0001 N: Dilute 0.0872 mL of concentrated hydrochloric acid to 100 mL with distilled water. Standardize the prepared solution with 0.01 N sodium carbonate solution.</p>
Sample preparation	Refer method FSSAI 01.001:2021 for sample preparation.
Method of analysis	<p>Test portion Preparation: Pipette 10.0 ± 0.1 mL of the prepared test sample into a pre-weighed conical flask. Re-weigh the flask and its contents recording the weights to the nearest 0.1 mg.</p> <p>Precipitation and Filtration</p> <ol style="list-style-type: none"> 1. Add 40 mL of TCA solution to the conical flask. Weigh the flask and its contents again to the nearest 0.1 mg. Swirl to mix, let the flask stand for approximately 5 min to allow the precipitate to settle. 2. Filter the contents of the flask through a Whatman filter paper Grade 1 and collect the entire filtrate in a clean, dry conical flask. The filtrate shall be clear and free of particulate matter. If it is not, repeat the process of precipitation of filtration with a new test portion. If duplicate tests are to be done, separate precipitations and filtrations should be carried out. 3. Swirl the filtrate to ensure the complete mixing. Pipette 20 mL of the filtrate into a 50 mL beaker and weigh. Pour the filtrate from the beaker into a Kjeldahl flask containing the appropriate amount of boiling aids, K_2SO_4, copper (II) sulfate solution and concentrated sulphuric acid as specified in the Macro Kjeldahl method for the determination of total nitrogen content in milk. Immediately re-weigh the empty beaker. <p>Digestion and Distillation:</p> <ol style="list-style-type: none"> 1. Continue with the digestion and distillation procedure as described in method for the determination of total nitrogen content in milk Macro Kjeldahl method. <p>Titration:</p> <ol style="list-style-type: none"> 1. Titrate the boric acid receiving solution with standard hydrochloric acid (0.01 N) to the first trace of pink colour. Take the burette reading to at least the nearest 0.05 mL. A lighted stir plate may aid visualization of the end point. <p>Blank Test:</p>

	1. Simultaneously carry out a blank test. Digest, distil and titrate a blank comprising about 0.1 g of sucrose and 16 ± 0.5 mL of TCA solution according to the procedure as given above.
Calculation with units of expression	<p>Calculate the nitrogen content, expressed as a percentage by mass using the following formula:</p> $W_n = \frac{1.4007 \times (V_s - V_B) \times N}{W_f \times W_m / (W_t - 0.065 W_m)}$ <p>Where,</p> <p>W_n = nitrogen (NPN) content of sample, expressed as a percentage by mass;</p> <p>V_s = volume in mL of the standard hydrochloric acid used for sample;</p> <p>V_B = volume in mL of the standard hydrochloric acid used for blank test;</p> <p>N = Normality of the standard hydrochloric acid expressed to four decimal places;</p> <p>W_m = mass of test portion in g, expressed to nearest 0.1 mg.</p> <p>W_f = mass of 20 mL filtrate in g, expressed to nearest 0.1 mg.</p> <p>W_t = mass of the test portion plus 40 mL of TCA solution in g, expressed to nearest 0.1 mg.</p> <p>Note: The factor 0.065 in the denominator assumes that milk contains about 3.5 % fat and 3.0% true protein (thus 0.035 + 0.030). The factor may need to be adjusted for other liquid dairy products e.g. concentrated or fractionated skim or whole milk products.</p> <p>Express the nitrogen content to four decimal places.</p>
Reference	ISO 8968-4/IDF 20-4:2001 - Milk - Determination of nitrogen content - Part 4: Determination of non-protein-nitrogen content. International Organization for Standardization, Geneva, Switzerland.
Approved by	Scientific Panel on Methods of Sampling and Analysis

B. CREAM

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	<h3>Sample Preparation for Cream</h3>		
Method No.	FSSAI 01.028:2021	Revision No. & Date	0.0
Scope	<p>This scope is for the preparation of sample of cream and similar kind of products such as reconstituted cream, recombined cream, prepared cream (pre-packaged liquid cream, whipping cream, cream packed under pressure, whipped cream, fermented/cultured/sour cream, acidified cream) and <i>malai</i>.</p>		
Sample preparation	<ol style="list-style-type: none"> 1. The preparation of cream sample depends upon its physical condition. If, at room temperature (25 ± 3 °C), the cream is thin to pour easily, mix by repeated inversion of the container. 2. If the cream is too thick, stir gently, taking care that the top and the bottom layers get well mixed. 3. It may not be possible to mix by gentle stirring if the cream is very thick and the fat is partly separated, or if on stirring, the cream becomes thick or fat separates. Under these circumstances, warm the cream sample to temperature between 30 to 40 °C in water-bath and, while cooling it to room temperature (25 ± 3 °C), shake the container gently or stir the contents at intervals. 4. Keep the container covered as much as possible to avoid loss of moisture by evaporation. 5. If the cream sample shows any abnormality, it should be recorded. If satisfactory mixing cannot be achieved, the sample should not be tested. 6. In the laboratory, the exposure of cream sample to temperature below freezing point should be avoided. 7. Cream samples should be protected from light, heat, contaminating odours and be kept in a cool place, at a storage temperature of 0 to 5 °C. <p>Note: Sour cream should not be warmed but should be thoroughly stirred.</p>		
Reference	<p>IS 3509: 1966 (Reaffirmed year 2018). Method of sampling and test for cream. Bureau of Indian Standards, New Delhi. IS 12333: 2017/ ISO 6731: 2010. Methods for determination of total solids content in milk, cream, and evaporated milk- reference method. Bureau of Indian Standards, New Delhi.</p>		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Determination of Fat in Cream

Method No.	FSSAI 01.029:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in cream and similar kind of products such as reconstituted cream, recombined cream, prepared cream (pre-packaged liquid cream, whipping cream, cream packed under pressure, whipped cream, fermented/cultured/sour cream, acidified cream) and malai.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 5. Sulphuric acid: Not flammable but highly reactive. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 6. Iso amyl alcohol: It is a highly flammable liquid. Keep away from heat/sparks/open flames/hot surfaces. Perform all operations under a fume hood. 		
Principle	<p>Fat content in cream sample can be estimated either by Gerber Method or Rose-Gottlieb method.</p> <p>The Gerber method is suitable as a routine or screening test. It is an empirical method and reproducible results can be obtained if procedure is followed correctly. The cream sample is mixed with sulphuric acid and iso-amyl alcohol in a special Gerber tube, permitting dissolution of the protein</p>		

	<p>and release of fat. The tube is centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the fat content of the cream sample. This method can be used for routine purposes.</p> <p>Rose-Gottlieb Method is a gravimetric method in which fat globule membrane of milk fat globules is ruptured by addition of ammonia to cream sample to liberate the fat. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.</p>
Apparatus/Instruments	<p>Refer apparatus requirement stated for milk (FSSAI 01.023:2021), along with following additional requirement.</p> <ol style="list-style-type: none"> 1. Analytical Balance (Readability 0.0001 g). 2. Cream butyrometer; 70 percent (ISI marked).
Materials and reagents	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021)
Preparation of Reagents	
Sample preparation	Refer method FSSAI 01.028:2021 for sample preparation.
Method of analysis	<p>Method 1: Gerber Method:</p> <ol style="list-style-type: none"> 1. Transfer 10 mL of Gerber sulphuric acid into cream butyrometer using automatic measure, taking care not to wet the neck of the butyrometer with the sulphuric acid. 2. Weigh 5 ± 0.01 g of sample into the butyrometer without soiling the neck, using any suitable form of support for the butyrometer on the balance. 3. Add about 6 mL of the hot water (70 °C) to the butyrometer; add 1 mL of amyl alcohol, preferably by use of an automatic dispenser. 4. Adjust the level of the contents to about 5 mm below the shoulder by further additions of hot water. 5. In no circumstances shall the amyl alcohol be added to the butyrometer before the cream. 6. Close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid. Keep in a water bath at 65 ± 2 °C for at least 3 min and not less than 10 min taking care to have casein particles if any to dissolve fully, and centrifuge for 5 min at 1100 rpm (approximately 300 g). 7. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. Allow the centrifuge to come to rest. 8. Remove the butyrometer tubes and place in water bath at 65 ± 2 °C for at least 3 min and not less than 10 min.

	<p>9. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key.</p> <p>10. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid.</p> <p>11. When readings are being taken hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye, and then read the butyrometer to the nearest half of the smallest scale division.</p> <p>Method 2: Rose-Gottlieb Method:</p> <ol style="list-style-type: none"> 1. Weigh accurately 1-2 g (depending on fat percentage) of cream into a 50 mL beaker, add 9 mL of 0.5% sodium chloride solution to disperse and transfer to fat extraction apparatus. 2. Add 1 mL of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used), mix and shake thoroughly. 3. Proceed for the extraction of the fat by Rose-Gottlieb method as prescribed for milk (Method No FSSAI 01.023:2021) starting with the step indication addition of 10 mL ethyl alcohol onwards.
<p>Calculation with units of expression</p>	<p>Method 1: Gerber Method: See the method of analysis.</p> <p>Method 2: Rose-Gottlieb Method:</p> $Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
<p>Reference</p>	<ol style="list-style-type: none"> 1. IS 3509: 1966 (Reaffirmed year 2018): Method of sampling and test for cream. Bureau of Indian Standards, New Delhi. 2. IS 1224 (Part II): 1977 (Reaffirmed Year 2018). Determination of fat by the Gerber method: Milk products. Bureau of Indian Standards, New Delhi.
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>


Detection of Presence of Thickeners in Cream


Method No.	FSSAI 01.029:2021	Revision No. & Date	0.0
Scope	This method is for the detection of presence of thickeners in cream and similar kind of products such as reconstituted cream, recombined cream, prepared cream (pre-packaged liquid cream, whipping cream, cream packed under pressure, whipped cream, fermented/cultured/sour cream, acidified cream) and malai.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalies as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 3. Iodine: It is not compatible with combustibles, strong bases, halogens and ethanol. It reacts violently or explosively with acetylene, acetaldehyde, metal hydrides and metal carbides. 4. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 5. Mercury: Avoid all unprotected contact with this product. Avoid generating vapour or mists. It forms toxic chemicals on contact with ammonia, oxidizing agents, metals (aluminium) etc. 6. Concentrated nitric acid: It is a corrosive chemical. It is highly reactive and a dangerous explosion hazard. It reacts violently and explosively with most metals and powdered metals (antimony, bismuth etc.), alkaline earth metals (magnesium, calcium etc.), and metal hydrides to form flammable and explosive hydrogen gas. 7. Picric acid: If necessary, apply water to the powder (misting it if possible). This will reduce dust formation and make it safe to handle. Picric acid forms sensitive salts with, and is therefore incompatible with alkalis, transition metal oxides and alkaline salts, and heavy metal oxides. 		
Principle	Addition of iodine solution in milk samples gives the appearance blue		

	colour indicating the presence of starch, which disappears when sample is boiled and reappears on cooling. Gelatine is also used as a thickener in cream. It is detected by Stokes' test, in which the sample is mixed with water and Stokes reagent and filtered. A saturated solution of picric acid is then added to the filtrate and the colour development is observed. Yellow precipitate is produced in the presence of gelatine, and cloudiness is observed when gelatine is present in smaller amounts.
Apparatus	<ol style="list-style-type: none"> 1. Test tube. 2. 250 mL volumetric flask. 3. Whatman filter paper. 4. Analytical Balance (Readability 0.01 g).
Chemicals	<ol style="list-style-type: none"> 1. Iodine. 2. Ethanol (98%). 3. Sodium hydroxide. 4. Sodium carbonate. 5. Mercury. 6. Concentrated nitric acid (Approximately 15.8 N). 7. Picric acid.
Preparation of reagents	<ol style="list-style-type: none"> 1. Iodine solution: Dissolve 2.6 g of iodine and 3 g of potassium iodide in sufficient quantity of water and make up to 200 mL. 2. Stokes' reagent: Dissolve mercury in twice its weight of concentrated nitric acid and dilute to 25 times the volume with water.
Sample preparation	Refer method FSSAI 01.028:2021 for sample preparation.
Method of analysis	<p>Detection of Starch:</p> <ol style="list-style-type: none"> 1. Take about 5 mL of cream in a test tube, bring to boiling condition and allow the test tube to cool to $(25 \pm 3 \text{ }^\circ\text{C})$. 2. Add 1-2 drops of iodine solution to the test tube. 3. Observe the colour. <p>Detection of Gelatin in Cream:</p> <ol style="list-style-type: none"> 1. Gelatin may be detected by Stokes' test. 2. Mix together 10 mL cream, 20 mL water and 20 mL of Stokes reagent. 3. To the filtrate add an equal volume of saturated picric acid solution. 4. Yellow precipitate is produced in presence of considerable amount of gelatin, smaller amounts are indicated by cloudiness.
Inference (Qualitative analysis)	<ol style="list-style-type: none"> 1. Detection of starch: Development of blue colour indicates presence of starch which disappears when sample is boiled and reappears on cooling. The limit of detection of method is 0.02%. 2. Detection of Gelatin in Cream: Yellow precipitate is produced in presence of considerable amount of gelatin, smaller amounts are indicated by cloudiness.

Reference	<ol style="list-style-type: none"> 1. IS 1479 (Part I): 2016. Methods of test for Dairy Industry – Rapid Examination of Milk. Bureau of Indian Standards, New Delhi. 2. AOAC 920.106 (Modified Method). 21st Edn. (2019). Official Method, Starch in Confectionery, last para. AOAC International, USA. 3. Pearson’s Composition and Analysis of foods, 9th Edn. 1991 page 559.
Approved by	Scientific Panel on Methods of Sampling and Analysis

C. DAHI AND YOGHURT

	Preparation of Sample of Dahi and Yoghurt		
Method No.	FSSAI 01.031:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of plain dahi, yoghurt, flavoured dahi, partly skimmed yoghurt, flavoured partly skimmed dahi, skimmed yoghurt and flavoured skimmed dahi.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Spatula / Spoon. 2. Mortar and pestle. 		
Sample preparation	<p>For plain dahi, yoghurt, partly skimmed yoghurt, skimmed yoghurt etc</p> <ol style="list-style-type: none"> 1. Bring the sample to room temperature (preferably 25 °C). 2. Mix the sample carefully by means of spatula or spoon using a rotary motion which passes from the lower layers to the surface layers of the sample so as to displace and mix them well. 3. To get a representative sample, particularly in case of a thick-set product, it may be necessary to pour out the whole of the contents in a mortar and mix it thoroughly until the mass is homogeneous. 4. Aliquots are weighed out from this well-mixed sample. <p>For products containing non-dairy ingredients such as fruits and vegetables and their products thereof, cereals and cereal products, coconut and coconut products, honey, chocolate, nuts, coffee, spices, condiments, culinary herbs etc.</p> <ol style="list-style-type: none"> 1. Bring the sample to room temperature (preferably 25 °C). 2. Homogenize it using an appropriate device, in order to facilitate the grinding and dispersion of fruits etc. 3. Aliquots are weighed out from this well-mixed sample. 		
Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of Fat in Dahi and Yoghurt		
Method No.	FSSAI 01.032:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in plain Dahi, yoghurt, flavoured dahi, partly skimmed yoghurt, flavoured partly skimmed dahi, skimmed yoghurt and flavoured skimmed dahi.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 5. Amyl alcohol: It is a highly flammable liquid. Keep away from heat/sparks/open flames/hot surfaces. Perform all operations under a fume hood. 		
Principle	Fat content of Dahi or yoghurt is estimated by Rose-Gottlieb procedure. In this gravimetric method, fat globule membrane of milk fat globules is ruptured by addition of ammonia to sample to liberate the fat. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.		
Apparatus/Instruments	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021).		
Materials and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.031:2021 for sample preparation.		
Method of analysis	Rose-Gottlieb Method: Refer method stated for analysis of fat content in milk (Method No FSSAI		

	01.023:2021).
Calculation with units of expression	Refer calculation stated for analysis of fat content in milk (Method No FSSAI 01.023:2021).
Reference	IS 1479 (Part II): 1961 (Reaffirmed Year 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Milk Solids-Not-Fat (SNF) in Dahi and Yoghurt

Method No.	FSSAI 01.033:2021	Revision No. & Date	0.0
Scope	This method is for the determination of milk solids-not-fat in plain dahi and yoghurt.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Zinc oxide: Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. 		
Principle	<p>In this method, total solids in given sample are estimated. Fat content is subtracted from total solids to calculate at SNF content in the sample. In cultured dairy products such as Dahi, total solids are determined after neutralization of developed acidity with alkali. In the following procedure (IDF 151: 1991), the water is evaporated from a test portion of the sample in the presence of zinc oxide at a temperature of 100 ± 2 °C in drying oven. Lactic acid (determined by titration method) content is determined separately in the sample and is added to the moisture determined in the drying step to compensate for the loss of water as a result of neutralizing the sample by means of zinc oxide.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Shallow flat-bottom dishes of aluminium, nickel, stainless steel. 2. Analytical Balance (Readability 0.0001 g). 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Hot air oven (forced air type), capable of being controlled at 100 ± 2 °C. 5. Flat bottom dishes of height 20 to 25 mm, diameter 50 to 75 mm, and made of appropriate material (for example stainless steel, nickel or aluminium), provided with well-fitting, readily removable lids. 6. Boiling water-bath. 7. Spoon or spatula. 		

	<ol style="list-style-type: none"> 8. Homogenizer for homogenizing fruit yogurts. 9. Porcelain or silica, 7-8 cm diameter, about 1.5 cm in height and provided with easily removable but closely fitting lids.
Materials and reagents	<ol style="list-style-type: none"> 1. Phenolphthalein indicator. 2. Sodium hydroxide. 3. Zinc oxide. 4. Ethanol.
Preparation of reagents	<ol style="list-style-type: none"> 1. Standard sodium hydroxide solution – 0.1 N 2. Phenolphthalein indicator solution: Dissolve 1g of phenolphthalein in 100 mL of ethanol (95%, v/v). Add 0.1N Sodium hydroxide solutions until one drop gives a faint pink coloration. Dilute with distilled water to 200 mL.
Sample preparation	Refer method FSSAI 01.031:2021 for sample preparation
Method of analysis	<p>Determination of total solids</p> <p>Method 1: Sodium hydroxide method</p> <ol style="list-style-type: none"> 1. Heat the clean, dry, empty dish and lid in oven maintained at 100 ± 2 °C for 1 h, cool in a desiccator and weigh. 2. Quickly weigh to the nearest 0.1 mg, 4-5 g of prepared sample of curd or dahi, replace the lid and weigh again. 3. Add 1-2 drops of phenolphthalein solution to the sample in the dish and neutralise with 0.1 N sodium hydroxide solution to a faint pink colour. 4. Note the volume of 0.1 N sodium hydroxide required to neutralise the sample. 5. Place the dish without lid on a boiling water bath until the water is removed from the sample. Wipe the under-surface of the dish and place in the oven maintained at 100 ± 2 °C, for 3 h. 6. Remove the dish along with the lid and cool in a desiccator and weigh. Continue heating and re-weighing at hourly intervals until the difference between successive weighing do not vary by more than 0.5 mg. 7. Deduct half weight of the 0.1 N sodium hydroxide added to neutralize the sample from the residue after drying and calculate total solids as for whole milk. <p>Method 2: Zinc oxide method</p> <ol style="list-style-type: none"> 1. Bring the sample to temperature of 20-25 °C. Mix the sample carefully by means of spatula using a rotatory motion which passes from the lower layers to the surface layer of the sample so as to displace and mix them well. 2. Heat an open dish containing approximately 2 g of zinc oxide, together with lid and a stirring rod for 1 h in the oven maintained at 100 ± 2 °C. 3. Transfer the dish along with lid and stirring rod to the desiccator, allow


	<p>it to cool to room temperature (at least 45 min) and weigh the dish with the stirring rod and lid to the nearest 0.1 mg.</p> <ol style="list-style-type: none"> 4. Move the zinc oxide to one side of the prepared dish by tilting. Place on the clear space approximately 1.0 g of prepared test sample, replace the lid on the dish with the stirring rod on top and weigh the dish to the nearest 0.1 mg. 5. Add 5 mL of water to the test portion and thoroughly mix the diluted test portion and zinc oxide. Spread the mixture evenly over the bottom surface of the dish. Heat the dish on the boiling water bath and continue heating for approximately 30 min with frequent mixing of the contents of the dish during the early stages of the drying so as to obtain maximum evaporation of liquid. 6. Remove the dish from the water bath and wipe its base to remove any water. Leave the stirring rod in the dish and then place it together with lid by its side, in the hot air oven (100 ± 2 °C) for 3 h. 7. After drying, cover the dish with its lid and immediately transfer to the desiccator. Allow the dish and contents to cool in the desiccator to room temperature (at least 45 min) and weigh to the nearest 0.1 mg. 8. Again, heat the dish and contents together with its lid for a further 1h, cover the dish and transfer to the desiccator. Allow to cool and weigh to the nearest 0.1 mg. repeat the process of reheating and weighing until the difference in mass between two successive weighing does not exceed 1 mg. Record the lowest weight. 9. In order to compensate for the loss of water as a result of neutralizing the yoghurt by means of zinc oxide, determine the titratable acidity (expressed as g of lactic acid per 100 g of product) of the sample. <p>Determination of fat content: Refer to method for determination of fat content in dahi and yoghurt (Method No FSSAI 01.031:2021)</p> <p>Determination of sucrose content (if added in flavoured product) Refer to method stated for analysis of sucrose content in condensed milk (FSSAI 01.051:2021)</p>
<p>Calculation with units of expression</p>	<p>Sodium hydroxide method:</p> $a = \frac{N \times T.V \times 40}{1000 \times 2}$ <p>Total solids percent (%) (m/m) = $\frac{100 (M_2 - a)}{M_1}$</p> <p>Where, N = Normality of Sodium hydroxide T. V = Titre value</p>

	<p>M_2 = Weight in g of residue left after drying M_1 = Weight in g of the prepared sample taken</p> <p>Zinc Oxide method: The total solids contents, expressed as percentage by mass, is equal to:</p> $= \left[\left(\frac{M_2 - M}{M_1 - M} \right) \times 100 \right] + 0.1 a$ <p>Where, M= is the mass, in g of the dish (including zinc oxide), lid and stirring rod. M_1 = is the mass, in g, of the dish (including zinc oxide), lid, stirring rod and test portion. M_2 = is the mass, in g, of the dish, lid stirring rod and dried test portion (including zinc oxide) a = is the mass, in g, of lactic acid as obtained in Step I. it is equal to 0.1 g per g of lactic acid content.</p> <p>For plain dahi/ yoghurt Milk SNF Content (%) = Total solids (%) - Fat (%)</p> <p>For Flavoured product Milk SNF Content (%) = Total milk solid (%) - Fat (%) – Added sugar (%)</p>
Reference	<ol style="list-style-type: none"> 1. IS 12333: 2017/ ISO 6731: 2010 (Modified). Milk, Cream and Evaporated Milk -Determination of Total Solid Content – Reference method. Bureau of Indian Standards, New Delhi. 2. IDF. (2005). IDF Standard 151, Yoghurt. Determination of total solid content, International Dairy Federation: Brussels. 3. Pearson’s Composition and Analysis of Foods, 9th Edn. Page 570.
Approved by	Scientific Panel on Methods of Sampling and Analysis


Determination of Titratable Acidity in Dahi and Yoghurt

Method No.	FSSAI 01.031:2021	Revision No. & Date	0.0
Scope	This method is for the determination of titratable acidity in dahi.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	A known quantity of sample is neutralized with standardized sodium hydroxide solution with phenolphthalein indicator. The amount of sodium hydroxide required is a function of the amount of natural buffering substances present in the product, and of developed or added acid or alkaline substances.		
Materials and reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Phenolphthalein indicator. 3. Analytical Balance (Readability 0.01 g). 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Standard sodium hydroxide solution – 0.1 N. 2. Phenolphthalein indicator: Dissolve 1.0 g of phenolphthalein in 100 mL of 95% ethanol, add 0.1 N Sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 mL. 		
Sample preparation	Refer method FSSAI 01.031:2021 for sample preparation		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 10 g of the prepared dahi/yoghurt sample in a suitable dish or basin. 2. Add 30 mL of warm water, add 1 mL of phenolphthalein indicator. 3. Shake well and titrate against standard NaOH solution (0.1N). 4. Complete the titration in 20 sec. Keep a blank by taking 10 g of prepared dahi/yoghurt sample diluted with 30 mL of water in another dish for comparison of colour. 		
Calculation with unis of expression	<p>Titratable acidity (as lactic acid)percent by mass = $\frac{9 AN}{w}$</p> <p>Where,</p> <p>A = Volume of standard Sodium hydroxide required for titration</p>		


	N = Normality of Standard Sodium hydroxide solution w = weight of the sample taken for test in g
Reference	IS 1166: 1986 (Reaffirmed year 2018). Specifications for condensed milk, partly skimmed condensed milk (Second Revision). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis


 FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <small>Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</small>	Determination of Protein in Dahi and Yoghurt		
Method No.	FSSAI 01.035:2021	Revision No. & Date	0.0
Scope	This method is for the determination of protein in dahi and yoghurt. The protein content is determined from the organic Nitrogen content by Kjeldahl method.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalies as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk and milk product (FSSAI 01.025/026:2021)		
Apparatus/Instruments			
Material and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.031:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 4-5 g of the prepared dahi/yoghurt sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube. 2. Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021). 		
Calculation with units of expression	Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021)		
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 		

	3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis


 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Detection of Starch in Dahi		
Method No.	FSSAI 01.036:2021	Revision No. & Date	0.0
Scope	This method is for the detection of starch in dahi.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 2. Iodine: It is not compatible with combustibles, strong bases, halogens and ethanol. It reacts violently or explosively with acetylene, acetaldehyde, metal hydrides and metal carbides. 3. Glacial acetic acid: Use with adequate ventilation. Keep away from heat. Do not breathe gas/fumes/ vapor/spray. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, alkalis. Citric acid: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Excessive intake of citric acid may cause erosion of the teeth. Causes respiratory tract irritation. Repeated exposure may cause sensitization dermatitis. 		
Principle	Addition of iodine solution in dahi samples gives the appearance blue colour indicating the presence of starch which disappears when sample is boiled and reappears on cooling.		
Apparatus/Instruments	Refer requirement stated for detection of starch in milk (FSSAI 01.003:2021).		
Materials and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.031:2021 for sample preparation.		
Method of analysis	Refer method prescribed for detection of starch in milk (FSSAI 01.003:2021)		
Inference (Qualitative analysis)	Appearance of blue colouration after addition of iodine solution indicates the presence of starch, which disappears when the sample is boiled and reappears on cooling.		
Reference	IS 1479 (Part I): 2016. Methods of test for Dairy Industry-Rapid examination of Milk. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

D. CHEESE AND CHEESE PRODUCTS

	Preparation of Sample of Cheese and Cheese Products		
Method No.	FSSAI 01.037:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of cheese (all varieties).		
Sample preparation	<ol style="list-style-type: none"> 1. Prior to analysis, remove the rind or smear or mouldy surface layer of the cheese, in such a way as to provide a sample representative of the cheese as it is usually consumed. 2. Grind or grate the sample by means of an appropriate device; mix the ground or grated mass quickly, and if possible, grind or grate a second time, and again mix thoroughly. 3. If the sample cannot be ground or grated, mix it thoroughly by intensive stirring and kneading. 4. Transfer the test sample to an air-tight container to await analysis, which should be carried as soon as possible after grinding. 5. If delay is unavoidable, take all precautions to ensure proper preservation of the sample, and to prevent condensation of moisture on the inside surface of the container. 6. The storage should be at 10 to 12 °C. 7. All sample preparation should be carried out in a manner which minimizes moisture loss. Such moisture loss will have the effect of increasing the apparent fat content. 		
Reference	IS 12758: 2005 (Reaffirmed year 2019)/ I.S.O 1735: 2014. Cheese and Processed cheese products- Determination of Fat content by gravimetric method – reference method. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of Moisture in Cheese		
Method No.	FSSAI 01.038:2021	Revision No. & Date	0.0
Scope	This method is for the determination of moisture in cheese (all varieties).		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The moisture content of cheese is the loss in mass, expressed as a percentage by mass when the product is heated in an air oven at 102 ± 2 °C to constant mass.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Flat-bottom dishes with lid: Dishes of nickel, aluminium or of other suitable metal not affected by boiling water, 70 to 80 mm in diameter and not more than 25 mm deep, provided with short glass stirring rod having a widened flat end. The dishes shall have lids which fit well and can readily be removed. 2. Hot air oven (forced air type), capable of being controlled at 102 ± 1 °C. 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Sand, which passes through 500 μ sieve and is retained by 180 μ sieve. It shall be prepared by digestion with concentrated hydrochloric acid, followed by thorough washing with water. It shall then be dried and ignited till it is dull red. 5. Analytical balance (Readability 0.0001 g). 		
Sample preparation	Refer method FSSAI 01.037:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Heat the flat-bottomed metal dish containing 20 g of prepared sand and a stirring rod, in hot air oven for about 1 h. Allow to cool in an efficient desiccator for 30 to 40 min. 2. Weigh accurately 3 g of the prepared sample of cheese into a flat-bottomed dish (with a cover) previously dried and weighed containing about 20 g of prepared sand and a stirring rod. 3. Saturate the sand by careful addition of a few drops of distilled water, and thoroughly mix the wet sand with the cheese sample by stirring with the glass rod, smoothing out lumps and spreading the mixture over the bottom of the dish. 		

	<ol style="list-style-type: none"> 4. Place the dish on a boiling water-bath for 20 to 30 min, then wipe the bottom of the dish. Transfer the dish containing the material, along with glass rod after uncovering in an oven maintained at 102 ± 1 °C for about 4 h. 5. After 4 h replace the lid, transfer the covered dish to the desiccator, allow it to cool to 25 ± 3 °C and weigh it accurately and quickly to the nearest 0.1 mg. 6. Heat the uncovered dish and lid in the oven at 102 ± 1 °C for further 1 h, replace the lid, allow the covered dish to cool to room temperature (25 ± 3 °C) in the desiccator and weigh it. 7. Repeat the process of drying, cooling and weighing, until the successive weighings do not differ by more than 0.5 mg. Record the weight.
Calculation with units of expression	$\text{Moisture \% by mass} = \frac{M_1 - M_2}{M_1 - M} \times 100$ <p>Where, M is mass in g, of the empty dish along with sand and glass rod; M₁ is initial mass in g of the dish with lid, glass rod, sand and test portion; M₂ is the mass in g of the dish with lid, glass rod, sand and dried test portion Express the results to the nearest 0.01% (m/m).</p>
Reference	IS 2785: 1979 (Reaffirmed year 2016). Specification for Natural cheese (Hard Variety), Processed Cheese, Processed Cheese Spread and Soft Cheese. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis


 FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <small>Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</small>	Determination of Fat (on dry matter basis) in Cheese by Reference Method		
Method No.	FSSAI 01.039:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in cheese (all varieties).		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 5. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 		
Principle	In this method fat is extracted from the sample by digesting the protein matrix using hydrochloric acid. The liberated fat is extracted using a combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. The addition of ethanol facilitates the passage of the fat from the aqueous phase to the solvents. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.		
Apparatus/Instruments	Refer requirement stated for analysis of fat content in milk.		
Materials and reagents	<ol style="list-style-type: none"> 1. Refer requirement stated for analysis of fat content in milk. 2. Concentrated hydrochloric acid (Approximately 11.6 N) 		
Preparation of reagents	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021).		


Sample preparation	Refer method FSSAI 01.037:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately 1-2 g of prepared sample in a 100 mL beaker. 2. Add 10 mL of concentrated hydrochloric acid and heat on a Bunsen burner boil gently, with shaking, either over a flame or in boiling waterbath, until all solid particles are dissolved. Cool to room temperature (25 ± 3 °C) in running water. Transfer the contents to the Mojonnier fat extraction flask. 3. Proceed for the extraction fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021). <p>Determination of moisture Refer method for determination of moisture in cheese (FSSAI 01.038:2021)</p>
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where, M_1 is the mass, in g, of the fat-collecting flask and extracted matter after drying M_2 is the mass, in g, of the empty fat-collecting flask M_3 is the mass, in g, of the fat-collecting flask used in the blank test after drying M_4 is the mass, in g, of the empty fat-collecting flask used in the blank test w is the weight of the sample in g Total Solids (%) = 100-moisture (%)</p> $Fat \% (on\ dry\ matter\ basis) = \frac{Fat\ (\%)}{Total\ Solids\ (\%)} \times 100$
Reference	IS 2785 -1979 (Reaffirmed 2016). Specification for Natural cheese (Hard Variety), Processed Cheese, Processed Cheese Spread and Soft Cheese. Bureau of Indian Standards, New Delhi
Approved by	Scientific Panel on Methods of Sampling and Analysis


 Determination of Fat (on dry matter basis) in Cheese by Gerber Method	
Method No.	FSSAI 01.040:2021 Revision No. & Date 0.0
Scope	This method is for the determination of fat in cheese (all varieties).
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sulphuric acid: Highly reactive. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Amyl alcohol: It is a highly flammable liquid. Keep away from heat/sparks/open flames/hot surfaces. Perform all operations under a fume hood.
Principle	Cheese is mixed with sulphuric acid and iso-amyl alcohol in a special Gerber tube, permitting dissolution of the protein and release of fat. The tubes are centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the fat content of the cheese sample.
Apparatus/Instruments	Refer apparatus requirement stated for milk (FSSAI 01.023:2021), along with following additional requirement. <ol style="list-style-type: none"> 1. Analytical Balance (Readability 0.0001 g). 2. Cheese butyrometer; 40 percent (ISI marked).
Materials and reagents	<ol style="list-style-type: none"> 1. Concentrated Sulphuric acid (Approximately 36.8 N). 2. Iso amyl alcohol (furfural free). It should have density between 0.807 to 0.809 g/mL at 27 °C.
Preparation of Reagents	Preparation of Gerber Sulphuric acid: Take required volume of water in a Pyrex flask (generally 100 mL of water is required for 900 mL of concentrated sulphuric acid) kept in a basin of ice-cold water. Carefully add the commercial sulphuric acid in small quantities at a time keeping the container sufficiently cold and mix gently. After cooling the flasks, check the specific gravity of Gerber acid with hydrometer and if necessary, adjust the Gerber acid to the correct specific gravity with addition of water or acid taking same precautions as before till specific gravity is in the range of 1.807 to 1.812 g/mL at 27 °C (or 1.815 to 1.820 g/mL at 20 °C). Store the prepared acid in a glass stoppered bottle to avoid absorption of water.
Sample preparation	Refer method FSSAI 01.037:2021 for sample preparation.
Method of analysis	1. Grate samples of hard cheese; grind samples of soft cheese. Mix

	<p>thoroughly.</p> <ol style="list-style-type: none"> 2. Measure 10 mL of Gerber sulphuric acid into a cheese butyrometer tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. 3. Add gently from the wash bottle sufficient warm water (30 to 40 °C) to form a layer about 6 mm deep on top of the acid, allowing the water to flow down the side of the bulb. 4. Weigh 3 ± 0.01 g of sample into the butyrometer without soiling the neck, using any suitable form of support for the butyrometer on the balance. 5. Add 1 mL of amyl alcohol, preferably by use of an automatic dispenser. Add warm water (30 to 40 °C) from the wash bottle until the butyrometer is filled to about 5 mm below the shoulder. 6. Close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid. Keep in a water bath at 65 ± 2 °C for at least 3 mins and not less than 10 mins, taking care to have casein particles if any to dissolve fully, and centrifuge for 5 min. at 1100 rpm (approximately 300 g). 7. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. 8. Allow the centrifuge to come to rest. Remove the butyrometer tubes and place in a water bath at 65 ± 2 °C for at least 3 min and not less than 10 min. 9. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key. 10. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid. <p>When readings are being taken hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye, and then read the butyrometer to the nearest half of the smallest scale division.</p> <p>Determination of Moisture Refer to method of determination of moisture in cheese (FSSAI 01.038:2021)</p>
Calculation with units of expression	<p>Total solids (%) = 100- moisture (%)</p> $\text{Fat \% (on dry matter basis)} = \frac{\text{Fat (\%)}}{\text{Total Solids (\%)}} \times 100$
Reference	<p>IS1224 (Part II): 1977 (Reaffirmed Year 2018). Determination of fat by the Gerber method: Milk products. Bureau of Indian Standards, New Delhi.</p>
Approved by	<p>Scientific Panel on Methods of Sampling and Analysis</p>

E. CHANNA/PANEER


 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	<h3>Preparation of Sample of Channa/Paneer</h3>		
Method No.	FSSAI 01.041:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of channa/paneer.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Spatula / Spoon. 2. Mortar and pestle. 3. Grating device. 		
Sample preparation	<ol style="list-style-type: none"> 1. Pass Channa sample through 8 mesh sieve three times or grate sample and mix thoroughly or grind to a uniform mass in a glass pestle and mortar. 2. Grate the paneer sample quickly through a suitable grater. Mix the grated sample thoroughly. 3. Transfer the grated sample to an air-tight container prior to analysis, which should be carried as soon as possible after grinding. 4. Keep sample in an airtight container until the time of analysis. If delay is unavoidable, take all precautions to ensure proper preservation of the sample, and to prevent condensation of moisture on the inside surface of the container. 5. The storage temperature should be below 10 °C. All sample preparation should be carried out in a manner which minimizes moisture loss. Such moisture loss will have the effect of increasing the apparent fat content. 		
Reference	IS 12758: 2005 (Reaffirmed year 2019)/ I.S.O 1735: 2014. Cheese and Processed Cheese products - Determination of fat content by gravimetric method - reference method. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 Determination of Moisture in Channa/Paneer	
Method No.	FSSAI 01.042:2021 Revision No. & Date 0.0
Scope	This method is for the determination of moisture in channa/paneer.
Caution	Follow all safety procedures and wear laboratory apron and safety goggles. <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage.
Principle	The moisture content of channa/paneer is the loss in mass, expressed as a percentage by mass when the product is heated in an air oven at $102 \pm 2 \text{ }^\circ\text{C}$ to constant mass.
Apparatus/Instruments	Refer method of analysis of moisture in cheese (FSSAI 01.038:2021)
Materials and reagents	
Preparation of reagents	
Sample preparation	Refer method FSSAI 01.041:2021 for sample preparation.
Method of analysis	Refer method of analysis of moisture in cheese (FSSAI 01.038:2021)
Calculation with units of expression	$\text{Moisture \% by mass} = \frac{M_1 - M_2}{M_1 - M} \times 100$ <p>Where, M is mass in g, of the empty dish along with glass rod; M₁ is initial mass in g of the dish with lid, glass rod and test portion; M₂ is the mass in g of the dish with lid, glass rod and dried test portion Express the results to the nearest 0.01% (m/m).</p>
Reference	<ol style="list-style-type: none"> 1. IS 2785: 1979 (Reaffirmed year 2016). Specification for Natural cheese (Hard Variety), Processed Cheese, Processed Cheese Spread and Soft Cheese. Bureau of Indian Standards, New Delhi. 2. IS 10484: 1983 (Reaffirmed year 2019). Specification for Paneer. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 Determination of Fat (on dry matter basis) in Channa/Paneer	
Method No.	FSSAI 01.043:2021 Revision No. & Date 0.0
Scope	This method is for the determination of fat in channa/paneer on dry matter basis.
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 3. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas.
Principle	In this method using fat is extracted from the sample by digesting the protein matrix using hydrochloric acid. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. The addition of ethanol facilitates the passage of the fat from the aqueous phase to the solvents. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results. Total solids are separately determined and final results of fat content in the sample is expressed on dry matter basis.
Apparatus/Instruments	Refer requirement stated for analysis of fat content in cheese (FSSAI 01.039:2021).
Materials and reagents	
Preparation of reagents	
Sample preparation	

Method of analysis	Refer method of analysis for determination of fat content in cheese (FSSAI 01.039:2021). Determination of moisture 1. Refer method for determination of moisture in channa/paneer FSSAI 01.042:2021.
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where, M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying M₂ is the mass, in g, of the empty fat-collecting flask M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test w is the weight of the sample in g</p> <p>Total Solids (%) = 100 - moisture (%)</p> $Fat \% (on\ dry\ matter\ basis) = \frac{Fat\ (\%)}{Total\ Solids\ (\%)} \times 100$
Reference	1. IS 2785: 1979 (Reaffirmed year 2016). Specification for Natural cheese (Hard Variety), Processed Cheese, Processed Cheese Spread and Soft Cheese. Bureau of Indian Standards, New Delhi. 2. IS 10484: 1983 (Reaffirmed year 2019). Specification for Paneer. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

F. ICE CREAM AND RELATED PRODUCTS


	Preparation of Sample for Ice cream, Kulfi, Milk Ices, Milk Lollies and Frozen Dessert/Confection		
Method No.	FSSAI 01.044:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of ice cream, kulfi, milk ices, milk lollies and frozen dessert/confection.		
Caution	Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.		
Sample preparation	<p>Preparation of Sample of Ice Cream: For samples taken in small packages, remove the packaging and place the sample in a clean, dry container fitted with an airtight closure. For samples taken from bulk or from large packages, keep them in their sampling containers.</p> <ol style="list-style-type: none"> 1. Plain ice cream: Allow the sample to soften at room temperature. It is not advisable to soften the ice-cream sample by heating on water bath or over flame because melted fat tends to separate and rise to the surface. After softening of ice-cream, mix it thoroughly by stirring with spoon or eggbeater or by pouring back and forth between beakers: 2. Ice cream containing fruits, nuts and chocolate ice-cream containing insoluble particles: Use a mixer capable of comminuting product to fine, uniform pulp. Use 100 - 200 g of ice-cream sample to fill the cup of mixer full to about one-third. Melt the product at room temperature (25 ± 3 °C) or in an incubator at 37 °C in a closed container. Transfer entire contents to the mixer cup and mix until insoluble particles are finely divided (about 3-5 min for fruit ice-cream and up to 7 min for nut ice-cream). Alternatively, the product may be grounded in porcelain or glass pestle and mortar. Transfer the mixed ice-cream sample to a suitable container for convenience in weighing. After weighing operation, return the remainder of the ice-cream sample to the refrigerator, preferably at a temperature not exceeding -15 °C. In case of ice cream, where the chocolate or similar covering portions forms a separate layer, it shall be removed and only the ice cream portion shall be taken for analysis. <ul style="list-style-type: none"> • Note: For determination of overrun in ice-cream, the entire ice-cream carton/cup should be taken as such. 		
Reference	<ol style="list-style-type: none"> 1. IS 2802: 1964 (Reaffirmed year 2016). Specification for Ice cream. Bureau of Indian Standards, New Delhi. 2. AOAC 969.20. 21st Edn. (2019). Official method. Ice cream and Frozen 		

	Desserts. AOAC International, USA.
Approved by	Scientific Panel on Methods of Sampling and Analysis


Determination of Total Solids in Ice Cream

Method No.	FSSAI 01.045:2021	Revision No. & Date	0.0
Scope	This method is for the determination of total solids in ice cream, kulfi, milk ices, milk lollies and frozen dessert/confection.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	In this method the sample is repeatedly heated and cooled till the difference between consecutive weighing agrees within 0.5 mg. Total solids is calculated from loss in weight observed.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Flat bottomed dishes of aluminium, nickel or stainless steel not affected by boiling water, 7-8 cm in diameter and not more than 2.5 cm deep provided with short stirring rods having a widened flat end. 2. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 3. Analytical balance (Readability 0.00) 4. 01 g). 		
Materials and reagents	Sand which passes through 500 micron I.S sieve and is retained on 180-micron sieve. It shall be prepared by digesting with concentrated hydrochloric acid followed by thorough washing with water. It shall be dried and ignited to red heat. (Refer clause 5.7 of IS 11622: 1986 for detailed procedure of preparation of sand)		
Preparation of reagents	<ol style="list-style-type: none"> 1. Quartz sand or sea sand, which passes through 500 microns IS sieve and is retained on 180 microns IS sieve [IS: 460 (Part I) – 1978], and which passes the suitability test size as follows: <ol style="list-style-type: none"> a. Place approximately 20 g of sand in a dish containing a stirring rod. Heat the open dish and sand, stirring rod and lid in a hot air oven controlled at 102 ± 2 °C, for at least 2 h or preferably overnight. Fit the lid, allow the dish to cool in a desiccator to room temperature (25 ± 3 °C) and weigh to the nearest 0.1 mg. b. Moisten the sand with approximately 5 mL of water, mix the sand and water using the stirring rod and heat the dish and sand, stirring rod and lid in a hot air oven, controlled at 102 ± 2 °C for at least 4 h. Fit the lid, 		

	<p>allow the dish to cool in the desiccator to room temperature (25 ± 3 °C) and weigh again to the nearest 0.1 mg. The difference between the two weighings shall not exceed 0.5 mg.</p> <p>If the requirements given above is not met, the sand may be made suitable for the determination as follows:</p> <p>Leave the sand immersed in 25% (m/m) hydrochloric acid solution for 3 days. Stir occasionally, decant off the supernatant liquid as far as possible, then wash the sand with water until the acid reaction has disappeared. Calcine (heat) the sand at 550 °C for at least 4 h using a muffle furnace. Repeat the test for the suitability of the sand as described above (the difference in weights represents the weight of fat extracted from the milk. Correct weight of extracted fat by blank determination on reagents used).</p>
Sample preparation	Refer method FSSAI 01.044:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Heat the moisture dish containing 20 g of prepared sand and glass stirring rod in the oven for 1 h; cool and weigh. 2. Weigh accurately about 5 g of sample into the dish. Add a few drops of water to assist in spreading the sample with glass rod. 3. Place dish on a boiling water bath for about 30 min. 4. Wipe bottom of dish and transfer to the air oven. Dry for about 4 h in an oven maintained at 102 ± 2 °C., remove dish to an efficient desiccator, allow to cool and weigh. 5. Replace dish in oven for a further period of 1 h, transfer to desiccator, allow it to cool and weigh. 6. Repeat the process of heating and cooling till consecutive weighing agree within 0.5 mg. Calculate total solids from loss in weight observed.
Calculation with units of expression	<p>Total Solids Content (%) = $\frac{M_2 - M}{M_1 - M} \times 100$</p> <p>Where,</p> <p>M is the mass in g of empty dish along with lid and sand and glass rod; M₁ is the initial mass in g of dish with lid, sand, glass rod and test portion M₂ is mass in g of dish with lid, sand and dried test portion Round the value obtained to nearest 0.01 % (m/m)</p>
Reference	<ol style="list-style-type: none"> 1. IS 2802: 1964 (Reaffirmed year 2016). Specification for Ice cream. Bureau of Indian Standards, New Delhi. 2. Pearson's Composition and analysis of foods, 9th Edn, 1991 page 604.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Weight per Unit Volume or Over-run in Ice Cream		
Method No.	FSSAI 01.046:2021	Revision No. & Date	0.0
Scope	This method is for the determination of weight per unit volume or over-run in ice cream.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Amyl alcohol: It is a flammable liquid. Keep away from heat, sparks and open flame. It is not compatible with strong acids (sulphuric acid, hydrochloric acid) and strong bases (Sodium hydroxide, potassium hydroxide). 		
Principle	Over-run is usually defined as the volume of ice-cream obtained in excess of the volume of the mix. It is usually expressed as a percentage. This increased volume is composed mainly of the air incorporated during the freezing process. The amount of air which is incorporated depends upon the composition of mix and the way it is processed. In this test, the volume of water and alcohol used corresponds with the volume of air originally contained in the ice-cream and the difference between the sum of these two and capacity of the flask is equivalent to the volume occupied by the sample.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Beaker: 400 mL. 2. Volumetric flask: 250 mL. 3. Glass funnel. 4. Analytical balance (Readability 0.0001 g). 		
Materials and reagents	n-Amyl alcohol (sp. gr. 0.817).		
Sample preparation	Refer method FSSAI 01.044:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh a unit of ice-cream and from it calculate the weight of ice-cream per litre. For example, 200 mL of a full carton of ice-cream can be obtained, the ice-cream carefully removed and the empty dry carton weighed. The difference in weights between the carton when filled and when empty is, therefore, the weight of 200 mL of frozen ice-cream. Five times this weight would then equal the weight of a litre. To determine the weight of the mix, proceed as below 2. Weigh and record the exact weight of a clean, dry 400 mL beaker. Into the beaker, weigh exactly 130 g of the frozen ice-cream. 3. Place the beaker in water bath warmed to 49 °C and melt. 4. Weigh and record the exact weight of a 250 mL volumetric flask. 5. Using a glass funnel, transfer 130 g of melted ice-cream into the 250 mL 		

	<p>volumetric flask.</p> <ol style="list-style-type: none"> 6. Add exactly 10 g of n-amyl alcohol to the flask and mix to break the surface tension of the melted ice-cream and release the incorporated air. 7. 10 g of n-amyl alcohol occupies a volume of 12.24 mL. 8. Cool the flask with contents to 15.5 °C using a cold water or ice water bath. 9. Rinse the beaker containing melted mix with several small rinsing of water, adding each rinse to the 250 mL flask. 10. Again cool the flask with contents to 15.5 °C and using the final rinse water, bring the volume to 250 mL mark. The bottom of the meniscus should correspond with the mark when temperature is exactly 15.5 °C. Dry the outside of the flask and reweigh. 11. Calculate the weight in g of the contents. Calculate the weight in g of the water added to the flask.
Calculation with units of expression	Calculate the volume in mL occupied by the sample of ice-cream. Determine the sp. gr. of the mix by dividing its weight (130 g) by the volume in mL, which it occupied. Determine the weight in g per litre of mix by multiplying by the specific gravity.
Reference	IS 2802: 1964 (Reaffirmed year 2016). Specification for ice-cream. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 Determination of Milk Fat in Ice cream	
Method No.	FSSAI 01.047:2021 Revision No. & Date 0.0
Scope	This method is applicable for the determination of milk fat in ice cream, kulfi, milk ices, milk lollies and frozen dessert/confection.
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: It is an extremely caustic liquid and gas. Wear skin, eye and respiratory protection while handling.; Perform all operations under a fume hood. 2. Ethyl alcohol: It is flammable. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: It is extremely flammable. Avoid breathing fumes. Perform all operations under a fume hood.
Principle	Sample is treated with ammonia and ethyl alcohol; the former to dissolve the protein and the latter to help precipitate the proteins. Fat is extracted with diethyl ether and petroleum ether. Mixed ethers are evaporated and the residue weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results. It may be noted that this method will determine total fat in the product and if any non-milk fat is present in the sample, the same will also be determined.
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Mojonnier fat extraction flask or any other suitable extraction tube (as per IS specification). 2. Cork or stopper of synthetic rubber unaffected by usual fat solvents. 3. 100 mL flat bottom flask with G/G joint or stainless steel or aluminium dishes of 5.5 cm height and 9 cm diameter or glass bowl. 4. Water bath. 5. Analytical balance (Readability 0.0001 g).
Materials and reagents	<ol style="list-style-type: none"> 1. Ammonia solution (Specific gravity 0.91). 2. Ethyl alcohol (95%). 3. Diethyl ether, peroxide-free. 4. Petroleum ether, boiling range 40 - 60 °C.
Sample preparation	Refer method FSSAI 01.044:2021 for sample preparation.
Method of analysis	Rose-Gottlieb Method:


	<ol style="list-style-type: none"> 1. Accurately weigh 4-5 g of the thoroughly mixed sample; transfer it directly into fat extraction flask or Mojonnier tube, using free flowing pipette. 2. Dilute with water to approximately to 10 mL, working the sample into the lower chamber and mix by shaking. 3. Add 2 mL ammonia; mix thoroughly, heat in water bath for 20 mins at 60 °C with occasional shaking. 4. Cool and proceed extraction of fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021).
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Reference	Pearson's Composition and analysis of foods 9 th edn, 1991 page 604.
Approved by	Scientific Panel on Methods of Sampling and Analysis


Determination of Protein in Ice-cream

Method No.	FSSAI 01.048:2021	Revision No. & Date	0.0
Scope	This method is for the determination of protein in ice cream, kulfi, milk ices, milk lollies and frozen dessert/confection. The protein content is determined from the organic Nitrogen content by Kjeldahl method.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 		
Principle	The various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with an alkali (Sodium hydroxide) and the ammonia liberated is absorbed in excess of standard solution of acid and then back titrated with standard alkali.		
Apparatus/Instruments	1. Refer the method for determination of protein by Kjeldahl method in milk and milk products (Refer method FSSAI 01.025/026:2021)		
Material and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.044:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 5-8 g of the prepared ice-cream sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube. 2. Refer the method for determination of protein in milk and milk products (FSSAI 01. 025/026:2021). 		
Calculation with units of expression	Refer the method for determination of protein in milk and milk products (FSSAI 01. 025/026:2021)		
Reference	1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for		


	<p>Standardization, Geneva.</p> <p>2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva.</p> <p>3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.</p>
Approved by	Scientific Panel on Methods of Sampling and Analysis

G. CONDENSED/EVAPORATED MILK

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	<h3>Preparation of Sample of Condensed/Evaporated Milk</h3>		
Method No.	FSSAI 01.049:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of condensed/evaporated milk.		
Sample preparation	<ol style="list-style-type: none"> 1. Shake the container thoroughly with frequent inversion. 2. Open the container and pour the milk slowly into another container made of glass or other suitable material provided with an air tight lid, taking care to incorporate in the sample any fat or other constituents adhering to the sides of the original container. 3. Stir vigorously and close the container. Alternatively heat the closed container in a water bath at 40-60 °C. 4. Remove and shake the container vigorously every 15 min. After 2 h, remove the container and cool to 20-25 °C. Remove the lid and mix thoroughly by stirring the milk with a spoon or spatula. <p>Note: If the fat separates, correct results cannot be obtained.</p>		
Reference	<ol style="list-style-type: none"> 1. IS 12333: 2017/ ISO 6731: 2010. Methods for determination of total solids content in milk, cream, and evaporated milk- reference method. Bureau of Indian Standards, New Delhi. 		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Fat in Condensed/Evaporated Milk		
Method No.	FSSAI 01.050:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in condensed/evaporated milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	Refer principle stated for analysis of fat content in milk (FSSAI 01.023:2021).		
Apparatus/Instruments	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021).		
Materials and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.049:2021 for sample preparation.		
Method of analysis	<p>Rose-Gottlieb Method</p> <ol style="list-style-type: none"> 1. Weigh accurately 2-2.5 g in case of condensed milk and 4 to 5 g for evaporated milk sample into a beaker. 2. Add 1 mL of water and make into a smooth paste by using a glass rod. 3. Add 9.0 mL of additional water and 1.25 mL of ammonium hydroxide and warm on a steam bath. 4. Transfer to Mojonnier flask, cool and proceed for the extraction fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021). 		
Calculation with units of expression	$\text{Fat \% (m/m)} = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$		

	<p>Where,</p> <p>M_1 is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M_2 is the mass, in g, of the empty fat-collecting flask</p> <p>M_3 is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M_4 is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Reference	<ol style="list-style-type: none"> 1. IS 1166: 1986 (Reaffirmed year 2018). Specification for condensed milk, partly skimmed condensed milk. Bureau of Indian Standards, New Delhi. 2. Pearson's Composition and analysis of foods 9th edn, 1991 page 604.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Sucrose Content in Condensed/Evaporated Milk		
Method No.	FSSAI 01.051:2021	Revision No. & Date	0.0
Scope	This method is for the determination of sucrose content in condensed/evaporated milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide solution: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ammonia solution: It reacts violently with halogens (such as fluorine, chlorine, bromine), acids (such as hydrochloric acid, hydrogen bromide, hydrogen fluoride). It is incompatible with cyanide and oxidizing agents. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 		
Principle	<p>Lane-Eynon (Volumetric) Method: The principle involves clarifying the sample with the help of zinc acetate and potassium ferrocyanide, inverting a part of the sample using hydrochloric acid and determining the reducing sugars content in both inverted and non-inverted parts of the sample. The sugar content is estimated by determining the volume of the unknown sugar solution required to completely reduce a measured volume of Fehling's solution. Invert sugar reduces the copper in Fehling's solution to red insoluble cuprous oxide.</p> <p>Polarimetric Method: The method based on Clerget inversion involves treatment of milk without acid which brings about complete hydrolysis of</p>		

	<p>sucrose, but almost none of lactose or other sugars. Sugar content is obtained from change in rotating power of the solution. A clear filtrate of sample, without mutarotation by lactose is prepared by treating the solution with ammonia followed by neutralisation and clarification with successive addition of zinc acetate and potassium ferrocyanide solution. In a part of the filtrate, the sucrose is hydrolysed by appropriate method and from the rotation of filtrate, before and after inversion, sucrose content is calculated.</p>
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Volumetric flasks: 100, 250, 500 and 1000 mL capacities. 3. Burette: 50 mL, graduated to 0.1 mL. 4. Funnels. 5. Conical flasks: 250 mL capacities. 6. Pipette: 1, 5 and 10 mL capacities. 7. Measuring cylinder: 25 and 50 mL capacities. 8. Whatman filter paper: Grade 1. 9. Watch glass. 10. Hot plate. 11. Polarimeter or saccharimeter. 12. Polarimeter tube: length 200 mm, of exactly calibrated length). 13. Water bath: Regulated at 60 ± 1 °C.
Materials and reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Sucrose. 3. Methylene blue indicator. 4. Fehling's solution. 5. Zinc acetate. 6. Potassium ferrocyanide. 7. Concentrated hydrochloric acid (Approximately 11.6 N). 8. Concentrated ammonia (Specific gravity 0.88). 9. Copper sulphate. 10. Concentrated sulphuric acid (Approximately 36.8 N). 11. Potassium sodium tartrate.
Preparation of Reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide solution: Approximately 0.1 N. Weigh 4 g of Sodium hydroxide and dissolve in 100 mL, cooling the flask. Make up the volume to 1000mL 2. Stock solution of invert sugar (Solution A): Weigh accurately 9.5 g of pure sucrose on a watch glass and transfer it to a one litre volumetric flask with 100 mL of water; add 5 mL of concentrated hydrochloric acid. Allow this to stand for 3 days at 20 to 25 °C and then make up to volume with water (this solution is stable for several months).

3. Standard solution of invert sugar (Solution B): Neutralise a known aliquot of the stock solution of invert sugar (Solution A) with sodium hydroxide solution using litmus paper and dilute with water to a known volume, so that more than 15 mL but less than 50 mL of it shall be required to reduce all the copper in the Fehling's solution taken for titration. Note the concentration of invert sugar in this solution as mg per 100 mL. Prepare this solution fresh every day.

Note: When 10 mL of Fehling's solution are taken for titration, a standard invert sugar solution containing 0.12 to 0.30 percent (m/v) of invert sugar is used. Usually 40 mL of Stock solution (Solution A) is diluted to 100 mL to get titration reading of around 15 mL when titrated against Fehling solution.

4. Methylene blue indicator solution: Dissolve 0.2 g of methylene blue in water and dilute to 100 mL.
5. Fehling's solution (Soxhlet modification): Prepared by mixing immediately before use, equal volumes of Solution A and Solution B prepared as described below.
6. Solution A: Dissolve 34.639 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water add 0.5 mL of concentrated sulphuric acid of sp. gr. 1.84 and dilute to 500 mL in a volumetric flask. Filter the solution through prepared asbestos.
7. Solution B: Dissolve 173 g of Rochelle salt (potassium sodium tartrate – $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) and 50 g of sodium hydroxide analytical reagent grade in water, dilute to 500 mL in a volumetric flask and allow the solution to stand for two days. Filter this solution through prepared asbestos.

Standardization of Fehling's Solution: Pipette accurately 10 mL of each Fehling's solutions (Solution A and B) into a 250 mL Erlenmeyer flask. Add about 10 mL of standard solution of invert sugar (Solution B) to the flask and heat to boiling. Boil for about 15 secs and add rapidly further portions of the standard solution of invert sugar until only the faintest perceptible blue colour remains, then add 2-5 drops of methylene blue solution, continue the heating and addition of Standard solution of invert sugar (Solution B) drop wise until the titration is complete, which is shown by the reduction of the dye (the end point of the titration is blue to red. Do not stir the flask, mixing is achieved by continuous boiling). Note down the volume of standard solution of invert sugar (Solution B) required to reduce all the copper. Repeat the titration, adding before heating almost all of the standard solution of invert sugar (Solution B) needed to reduce all the copper so that not more than 0.5-1 mL is required later to complete the

	<p>titration. Heat the mixture to boiling and boil gently for 2 min, lowering the flame sufficiently to prevent bumping. Without removing the flask from the flame add 2-5 drops of methylene blue indicator and complete the titration within a total boiling time of about 3 min, by small additions of standard solution of invert sugar (Solution B) to complete decolourization of the indicator. Note down the volume of standard solution of invert sugar (Solution B) required to reduce all the copper.</p> <p>Note 1: The concentration of the sugar solution should be such that the titre volume should be between 15 and 50 mL and this can be achieved with 0.2 -0.3 g sugar per 100 mL.</p> <ol style="list-style-type: none"> 8. Zinc acetate solution: Dissolve 21.9 g of crystalline Zinc acetate in water and add 3 mL of glacial acetic acid; make volume up to 100 mL. 9. Potassium ferrocyanide solution: Dissolve 10.6 g of crystalline Potassium ferrocyanide and make up to 100 mL with water. 10. Concentrated hydrochloric acid (Approximately 11.6 N) 11. Dilute hydrochloric acid: 6.35 N \pm 0.2 (20-22 %); required for polarimetric method only. 12. Concentrated ammonia solution (Specific gravity 0.88). 13. Dilute ammonia solution: 10 mL of concentrated ammonia solution diluted to 100 mL with distilled water. 14. Dilute acetic acid solution: 10 mL of acetic acid solution diluted to 100 mL with distilled water.
<p>Method of analysis</p>	<p>Method 1. Lane-Eynon (Volumetric) Method:</p> <p>Preparation of the Solution:</p> <ol style="list-style-type: none"> 1. Weigh accurately about 40 g of the well-mixed sample and transfer to 100 mL beaker. 2. Add 50 mL of hot water at 80 to 90 °C. Mix and transfer to a 250 mL measuring flask washing it with successive quantities of distilled water at 60 °C, until the volume is 120 to 150 mL. 3. Mix and cool to room temperature (25 \pm 3 °C) and add 5 mL of the dilute ammonia solution. Mix and allow to stand for 15 min. Add 5 mL of dilute acetic acid to neutralize the added ammonia. This can be confirmed by placing a drop of solution on a blue litmus paper. The litmus paper should turn red. Mix and add 12.5 mL of zinc acetate solution followed by 12.5 mL of potassium ferrocyanide solution; mix again. 4. Make up to 250 mL mark, allow it to settle and filter. Mark this solution as B-I. 5. Pipette 50 mL of solution B-I into a 100 mL volumetric flask, add 5

mL of concentrated hydrochloric acid and heat in a water bath at 65-68 °C for 5 min rotating the flask for the first 3 min.

6. Cool the solution and neutralize with sodium hydroxide solution. Mark this solution as A- I, and make up the volume to 100 mL.
7. Dilute the solutions B-I (usually 50 mL of B-I solution is required to be diluted to 100 mL to obtain titration reading of around 14 mL when titrated against Fehling solution) and A-I (usually 15 mL of A- I solution is required to be diluted to 100 mL to obtain titration reading of around 18 mL when titrated against Fehling solution) so that the volume of solution required to react with 10 mL Fehling's solution is between 15- and 50-mL. Mark them B-II and A-II, respectively.

Incremental Method of Titration

1. Pour the prepared solution into a 50 mL burette (see Note 6). Pipette 10 mL of mixed Fehling's solution into a 250 mL conical flask and run in from the burette 15 mL of the solution.
2. Without further dilution, heat the contents of the flask over a wire gauze, and boil (after the liquid has been boiling for about 15 sec, it will be possible to judge if the copper is almost fully reduced by the bright red colour imparted to the boiling liquid by the suspended cuprous oxide. When it is judged that nearly all the copper is reduced, add 1 mL of methylene blue indicator solution (see Note 2).
3. Continue boiling the contents of the flask for 1-2 min from the commencement of bubbling (boiling), and then add the prepared solution in small quantities (1 mL or less at a time), allowing the liquid to boil for about 10 sec between successive additions, till the blue colour of the indicator just disappears (see Note 5).
4. In case there appears to be still much unreduced copper, after the mixture of Fehling's solution with 15 mL of the prepared solution has been boiling for a 15 sec, add the prepared solution from the burette in larger increments (more than 1 mL at a time according to judgement), and allow the mixture to boil for a quarter of a min after each addition.
5. Repeat the addition of the prepared solution at intervals of 15 sec until it is considered safe to add a large increment of the prepared solution. At this stage, continue the boiling for an additional 1-2 min, add 1 mL of methylene blue indicator solution and complete the titration by adding the prepared solution in small quantities (less than 1 mL at a time) (see also Note 3).

Note 2: It is advisable not to add the indicator until the end point has been

reached, because the indicator retains its full colour until the end point is almost reached and thus gives no warning to the operator to go slowly.

Note 3: When the operator has had a fair amount of experience with the method, a sufficiently accurate result may often be obtained by a single estimation by the incremental method of titration, but for the utmost degree of accuracy of which the method is capable second titration should be carried out by the standard method of titration.

Standard Method of Titration

1. Pipette 10 mL of Fehling solution into a 250 mL conical flask and run in from the burette almost the whole of the prepared solution of B-II required to effect reduction of all the copper so that, if possible, not more than 1 mL shall be required later to complete the titration.
2. Gently boil the contents of the flasks for 2 min, at the end of 2 min of boiling add, without interrupting boiling, 1 mL of methylene blue indicator solution.
3. While the contents of the flask continue to boil, begin to add the prepared solution (1 or 2 drops at a time), from the burette till the blue colour of the indicator just disappears (see Note 4).
4. The titration should be completed within 1 min, so that the contents of the flask boil altogether for 3 min without interruption.

Note 4: The indicator is so sensitive that it is possible to determine the end point within one drop of the prepared solution in many cases. The complete decolourization of the methylene blue is usually indicated by the whole reaction liquid in which the cuprous oxide is continuously generated up becoming bright red or orange in colour. In case of doubt, the flame may be removed from the wire gauze for 1 or 2 sec and the flask held against a sheet of white paper. (A holder of paper, suitably affixed round the neck of the flask, is very convenient for this purpose as it can be left round the neck of the flask without risk of over-balancing it). The top edge of the liquid would appear bluish if the indicator is not completely decolourized. It is inadvisable to interrupt the boiling as the indicator undergoes back oxidation rather rapidly when air is allowed free access into the flask, but there is no danger of this as long as a continuous stream of steam is issuing from the mouth of the flask.

Note 5: It should be observed that with both incremental and standard methods of titration, the flask containing the reaction mixture is left on the wire gauze over the flame throughout the titration, may be removed for a few sec to ascertain if the end point is reached.

Note 6: In adding sugar solution to the reaction mixture the burette may

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

Method 2. Polarimetric Method:

Preparation of Sample

A. Samples of recently manufactured product in which no appreciable separation of components may be expected: Open the container, transfer all material adhering to the lid into the container and thoroughly mix by an up and down movement of a spoon so that the top layers and the contents of the lower corners are intermixed. Transfer the contents of can to a jar with well-fitting lid.

B. Samples of older products and samples in which separation of components may be expected: Heat in a water bath at about 40 °C until the sample has nearly reached this temperature, open the container and proceed as described above. When the product is in a can, transfer the contents to a jar, scrap out all material adhering to the walls and continue the mixing until the whole mass is homogeneous. Close the jar with a well-fitting lid.

Control Determination

Run a control determination as described under "Determination" in duplicate in a mixture of 100 g of milk (or 110 g of skim milk) and of pure sucrose (a) 18 g for 40 g of condensed milk (thus containing 45% sucrose). Calculate the sugar contents by means of the formula given at the end of this procedure using formula (1) for m, F and P respectively, which are the quantity of milk weighed and the fat and protein content of this milk, and in formula (2) for m, the value of 40. The mean of the values found shall not differ by more than 0.1 percent from the actual 45 percent.

Procedure:

1. Weigh approximately 40 g of the well mixed sample to an accuracy of 10 mg, into glass beaker.
2. Add 50 mL of hot distilled water (80-90 °C) and mix well.
3. Transfer the mixture quantitatively to 200 mL volumetric flask, rinsing the beaker with successive quantities of distilled water at 60 °C, until the total volume is between 120-150 mL.
4. Mix and cool, add 5 mL of ammonia solution, mix again and then

	<p>allow to stand for 15 min.</p> <ol style="list-style-type: none"> Neutralize the ammonia by adding equivalent quantity of acetic acid, having determined beforehand the exact number of millilitres by titration of the ammonia solution with bromothymol blue as indicator. Mix and add with gentle mixing, by rotating the tilted flask, 12.5 mL of zinc acetate solution. In the same manner add 12.5 mL of potassium ferrocyanide solution. Bring the contents of the flask to 20 °C and add water up to 200 mL mark. <p>Note: Up to this stage, all additions of water or reagents is made so as to avoid the formation of air bubbles, and with the same object in view, all mixing should be done by rotation of the flask rather than by shaking. If air bubbles are found to be present before diluting to 200 mL, their removal can be done by temporarily connecting the flask to a vacuum pump and rotating the flask; close the flask with a dry stopper and mix thoroughly by swirling. Let it stand for a few min and filter through a dry filter paper, rejecting first 25 mL of filtrate.</p> <p>Direct Polarisation: Determine optical rotation of filtrate at 20 ± 2 °C.</p> <p>Inversion:</p> <ol style="list-style-type: none"> Pipette 40 mL of the filtrate obtained above into the 50 mL volumetric flask and add 6.0 mL of diluted hydrochloric acid. Place the flask in the water bath at 60 °C for 15 min, the entire bulb of the flask being immersed. Mix by the rotary movement during the first 5 min; by this time the contents of the flask should have attained the temperature of the bath. Cool to 20 °C and make up to the 50 mL mark with distilled water at 20 °C. Mix, allow to stand for 1 h at this temperature. <p>Invert Polarization: Determine the rotation of the invert solution at 20 ± 2 °C. (When temperature of the liquid in the polarization tube differs by more than 0.2 °C during the measurement, the temperature correction referred to under Note 2 shall be applied).</p>
<p>Calculation with units of expression</p>	<p>Original Reducing sugars % (ORS) in condensed milk =</p> $\frac{W_1}{V_1} \times 250 \times \frac{100}{W_2} \times \frac{1}{1000} \times F_1$ <p>Total Reducing sugars % (TRS) in Condensed Milk = $\frac{W_1}{W_2} \times 25 \left[\frac{2F_2}{V_2} \right]$</p>

Sucrose % = TRS -ORS

Note: If standardization of Fehling solution is carried out by Lactose Solution (Reagent L) instead of Standard solution of invert sugar (Solution B) prepared under acidic conditions at room temperature (25 ± 3 °C), multiply the Sucrose % obtained in above formula by a factor 0.95 for calculating sucrose content.

Where,

W_1 = weight in mg of sucrose corresponding to 10 mL of Fehling's solution

W_2 = weight in g of the material taken for the determination

F_2 = dilution factor for solution A - II from A - I

V_2 = volume in mL of solution A - II corresponding to 10 mL of Fehling's solution

F_1 = dilution factor for the solution B - II from B - I

V_1 = volume in mL of solution B - II corresponding to 10 mL of Fehling's solution

Derivation of above formula

1. Standardization of Fehling Solution using Standard Reducing Sugar

Let 1 mL of Standard solution of invert sugar (Solution B) contains = M mg of Total Invert Sugar

2. Calculation of Lactose (original reducing sugar in SCM sample) estimation

Let V_1 = mL of diluted sample filtrate (**B1**) required for the titration of 10 mL of Fehling's solution

Let V = mL of the Standard solution of invert sugar (**Reagent C**) required for the titration of 10 mL of Fehling's solution

Now

10 mL of Fehling's solution = V mL of standard reducing sugar solution
= V_1 mL of diluted sample filtrate

Thus

V_1 mL of diluted sample filtrate \equiv V mL of standard reducing sugar solution
 $\equiv V \times M$ mg of standard reducing sugar

or $\equiv W_1$ mg of standard reducing sugar

Therefore, 100 mL of sample filtrate contains = $\frac{W_1}{V_1} \times 100$ mg of original reducing sugar

Since 100 mL of this sample filtrate was obtained from 50 mL of B1 solution

Therefore, 250 mL of B1 sample filtrate will contain = $\frac{W_1}{V_1} \times \frac{100}{50} \times 250$
mg of original reducing sugar

Since 250 mL of sample filtrate was obtained from 40 g (say W_2 g) of SCM
Therefore, Lactose (g) present in 100 gm of SCM (say C_1) =

$$\frac{W_1}{V_1} \times \frac{100}{50} \times 250 \times \frac{100}{W_2} \times \frac{1}{1000} g$$

2. Calculation for Total reducing sugar (Lactose + hydrolyzed Sucrose) in given sample

Let V_2 = mL of diluted sample filtrate (A1) required for the titration of 10 mL of Fehling's solution

Let V = mL of the Standard solution of invert sugar (Solution B) required for the titration of 10 mL of Fehling's solution

Now

10 mL of Fehling's solution = V mL of standard reducing sugar solution
= V_2 mL of diluted sample filtrate

Thus

V_2 mL of diluted sample filtrate $\equiv V$ mL of standard reducing sugar solution
 $\equiv V \times M$ mg of standard reducing sugar
or $\equiv W_1$ mg of standard reducing sugar

Therefore, 100 mL of sample filtrate (A-II) contains = $\frac{W_1}{V_2} \times 100$ mg of
total reducing sugar

Since 100 mL of this sample filtrate was obtained from 15 mL of A1 solution

Therefore, 100 mL of A-I sample filtrate will contain = $\frac{W_1}{V_2} \times \frac{100}{15}$ mg
of total reducing sugar

Since 100 mL of this sample filtrate was obtained from 50 mL of B1 solution

Therefore, 100 mL of B-I sample filtrate will contain = $\frac{W_1}{V_2} \times \frac{100}{15} \times \frac{100}{50}$
mg of total reducing sugar

Therefore, 250 mL of B1 sample filtrate will contain =
 $\frac{W_1}{V_2} \times \frac{100}{15} \times \frac{100}{50} \times 250$ mg of total reducing sugar

Since 250 mL of sample filtrate was obtained from 40 g (say W_2 g) of SCM
Therefore, Total reducing sugars (g) present in 100 g of SCM (say C_2) =

$$\left[\frac{W_1}{V_2} \times \frac{100}{15} \times \frac{100}{50} \times 250 \times \frac{100}{W_2} \times \frac{1}{1000} \right]$$

This calculation is on the assumption that 15 of A-1 solution was diluted to 100 mL

Total sucrose (%) in the SCM = $C_2 - C_1$ g

Polarimetric Method: Calculate the sucrose content S , percent by mass, by means of the following formula.

$$U = \frac{m}{100} (1.08 F + 1.55 P) - \text{Formula 1}$$

$$S = \frac{D - \frac{5}{4} I}{Q} \times \frac{V - U}{V} \times \frac{V}{e \times m} - \text{Formula 2}$$

Where,

U = Correction in mL for the volume of the precipitate formed during the clarification

m = Mass in g of the weighed sample

F = Percentage of fat in the sample

P = Percentage of protein ($N \times 6.38$) in the sample

D = Direct polarimeter reading (polarization before inversion)

I = Polarimeter reading after inversion

Q = Inversion division factor, the values of which are given in Note 1


V = Volume in mL to which the sample is diluted before filtration; and

e = Length in mm of the polarimeter tube.

Remarks:


1. When exactly 40 g of the condensed milk are weighed and a polarimeter with sodium light, angular degrees and a 2 dm polarimeter tube at 20.0 ± 1 °C is used the sucrose content of normal condensed milk ($C = 9$) can be calculated from the following formula; $S = (D - \frac{5}{4} I) 2.833 - 0.006 I 2F - 0.08 78P$.
2. If the invert polarisation is measured at a temperature other than 20 °C, the figures should be multiplied by: $1 + 0.0037 (T-20)$ Values of the inversion division factor (Q) -The following formula give accurate values for Q , for various sources of light with corrections, where necessary for concentration and temperature:
3. Sodium light and polarimeter with angular degrees: $Q = 0.8825 + 0.0006 (C-9) - 0.0033 (T-20)$.
4. Mercury green light and polarimeter with saccharimeter with international sugar scale degrees:

	<p>$Q = 2.549 + 0.0017 (C-9) - 0.0095 (T-20)$</p> <p>In the above formula: C = Percentage of total sugars in the invert solution as polarised; and T = Temperature of the invert solution in the polarimetric reading.</p> <p>Note 1: The percentage of total sugar C in the inverted solution may be calculated from the direct reading and the change on inversion in the usual manner, using the usual values for the specific rotations of sucrose, lactose, and invert sugar. The correction 0.0006 (C-9), etc., is only accurate when C is approximately 9. For normal condensed milk, this correction can be neglected C being close to 9.</p> <p>Note 2: Variation in temperature from 20 °C makes little difference in direct reading, but variation of over 0.2 °C in the invert reading necessitates a correction. The correction 0.0037 (T-20) etc. is accurate only between 18 and 22 °C.</p> <p>Repeatability (Polarimetric method): The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst in the same laboratory shall not exceed 0.3 g of sucrose per 100 g of condensed milk.</p>
Reference	<ol style="list-style-type: none"> 1. IS 4079: 1967 (Reaffirmed year 2016). Specification for canned rasogolla. Bureau of Indian Standards, New Delhi. 2. IS 11764: 2005 (Reaffirmed year 2019). Method for determination of sucrose content by Polarimetric method in condensed milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

		Determination of Milk Solids in Condensed/Evaporated Milk	
Method No.	FSSAI 01.052:2021	Revision No. & Date	0.0
Scope	This method is for the determination of milk solids in condensed/evaporated milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. <p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	In this method, first total solids are determined in the sample. In case of evaporate milk, total solids calculated in the sample are milk solids (%). In case of condensed milk, % sucrose is also estimated separately and milk solids are calculated by subtracting % sucrose from % total solids.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Flat-bottomed dishes: Made of aluminum alloy, nickel, stainless steel, porcelain, 50 to 75 mm diameter, 20 to 25 mm depth, and provided with easily removable but closely fitting lids. 3. Boiling water-bath. 4. Water-bath: Maintained at 40 – 60 °C. 5. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 6. Desiccator with efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 7. Short glass stirring rods: Flattened at one end and fitting in to the dish. 		


Materials and reagents	<ol style="list-style-type: none"> 1. Concentrated hydrochloric acid (Approximately 11.6 N). 2. Quartz sand or sea sand.
Preparation of reagents	<ol style="list-style-type: none"> 1. Quartz sand or sea sand, which passes through 500 microns IS sieve and is retained on 180 microns IS sieve [IS: 460 (Part I) – 1978], and which passes the suitability test size as follows: <ol style="list-style-type: none"> a) Place approximately 20 g of sand in a dish containing a stirring rod. Heat the open dish and sand, stirring rod and lid in a hot air oven controlled at 102 ± 2 °C, for at least 2 h or preferably overnight. Fit the lid, allow the dish to cool in a desiccator to room temperature (25 ± 3 °C) and weigh to the nearest 0.1 mg. b) Moisten the sand with approximately 5 mL of water, mix the sand and water using the stirring rod and heat the dish and sand, stirring rod and lid in a hot air oven, controlled at 102 ± 2 °C for at least 4 h. Fit the lid, allow the dish to cool in the desiccator to room temperature (25 ± 3 °C) and weigh again to the nearest 0.1 mg. The difference between the two weighing shall not exceed 0.5 mg. <p>If the requirements given above is not met, the sand may be made suitable for the determination as follows: Leave the sand immersed in 25% (m/m) hydrochloric acid solution for 3 days. Stir occasionally, decant off the supernatant liquid as far as possible, then wash the sand with water until the acid reaction has disappeared. Calcine (heat) the sand at 550 °C for at least 4 h using a muffle furnace. Repeat the test for the suitability of the sand as described above (the difference in weights represents the weight of fat extracted from the milk. Correct weight of extracted fat by blank determination on reagents used).</p>
Sample preparation	Refer method FSSAI 01.049:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Heat a dish containing about 20 g of the sand with its lid alongside and a stirring rod on top of the lid, in a hot air oven at $102^{\circ} \pm 2$ °C for about 1 h. 2. Place the lid (with the stirring rod on the top) on the dish, immediately transfer the dish to the desiccator and allow to cool for at least 45 min, and weigh the dish with lid and rod to the nearest 0.1 mg. 3. Tilt the sand to one side of the prepared dish, and place 2.0 g of the prepared test sample of condensed milk on the clear space, replace the lid with the stirring rod on top and weigh the dish to the nearest 0.1 mg. 4. Add 5 mL of distilled water to the test portion in the dish and mix with the stirring rod. Thoroughly mix together the diluted test portion and the sand, and spread the mixture evenly over the bottom of the dish. Leave the stirring end of the rod in the mixture with the other end resting on the rim of the dish.

	<ol style="list-style-type: none"> 5. Heat the dish on a boiling water-bath with the bottom of the dish exposed to steam, for approximately 30 min while stirring the mixture frequently in the early stages of drying so that the mixture is well aerated and becomes crumbly. 6. Lay the stirring rod flat inside the dish; dry the bottom of the dish and heat the dish with its lid alongside, in a hot air oven maintained at 102 ± 2 °C for 4 h. 7. Place the lid on the dish, allow the dish to cool in the desiccator and weigh to the nearest 0.1 mg. Repeat the operations described above (heating the dish for 1 h) until the difference in mass between two successive weighing does not exceed 0.5 mg. 8. Record the lowest mass. 9. In case of sweetened condensed milk, determine the sucrose (Refer method FSSA.01.054:2021).
Calculation with units of expression	<p>For evaporated milk</p> $\text{Total solids \% by mass} = \frac{M_2 - M}{M_1 - M} \times 100$ <p>Where M = mass in g, of the dish, lid, sand and stirring rod; M₁ = mass in g, of the dish, lid, stirring rod, sand and test portion; and M₂ = in g, of the dish, lid, stirring rod and dried test portion. Note: BIS/ISO method does not recommend use of sand. However, use of sand helps in evenly spreading the samples.</p> <p>For sweetened condensed milk</p> Milk solids (%) = Total solids (%) – Sucrose (%)
Reference	IS 11622: 1986. Method for determination of total solids content in condensed milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Milk Protein in Milk Solids-not-Fat of Condensed/Evaporated Milk		
Method No.	FSSAI 01.053:2021	Revision No. & Date	0.0
Scope	This method is for the determination of milk protein in milk solids not fat of condensed/evaporated milk.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk		
Apparatus/Instruments	and milk products (FSSAI 01.025/026:2021)		
Materials and reagents			
Preparation of Reagents			
Sample preparation	Refer method FSSAI 01.049:2021 for sample preparation.		
Method of analysis	Determination of Total Nitrogen in Sample <ol style="list-style-type: none"> 1. Weigh quickly about 1-2 g of the prepared condensed/evaporated milk sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube 		

	<p>taking care to see that no portion of the sample clings to the neck of the flask/tube.</p> <p>2. Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021).</p> <p>Determination of fat Refer the method for determination of fat in condensed/evaporated milk (FSSAI 01.050:2021).</p> <p>Determination of milk solids Refer the method for determination of milk solids in condensed/evaporated milk (FSSAI 01.052:2021).</p>
Calculation with units of expression	<p>Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021).</p> <p>Milk SNF (mSNF) Content (%) = Milk Solids (%) - Fat (%)</p> $\text{Protein on mSNF basis (\%)} = \frac{\text{Protein (\%)} \times 100}{mSNF}$
Reference	AOAC 991.23. 21 st Edn. (2019). Official Method 991.23 Protein Nitrogen content of Milk. AOAC International, USA.
Approved by	Scientific Panel on Methods of Sampling and Analysis


H. DRIED MILK PRODUCTS

	Preparation of Sample for Dried Milk Products		
Method No.	FSSAI 01.054:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample dried milk products such as Whole, Skimmed, Partially Skimmed Milk Powder, Cream Powder, Infant Milk Food, Infant Formula, Milk Cereal Weaning Food, Processed Cereal Based Weaning Food, Milk Protein Concentrates, Dairy Permeates, Colostrum Powder, and Dairy Whitener.		
Sample preparation	<ol style="list-style-type: none"> 1. Make the sample homogeneous either by mixing or shaking or alternately rolling and inverting the container. 2. Avoid excessive temperature and humidity when opening sample container to prevent absorption of moisture. These products are hygroscopic in nature. Care must be taken while handling these samples to avoid moisture absorption, which can give erroneous results. 		
Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Determination of Moisture in Dried Milk Products

Method No.	FSSAI 01.055:2021	Revision No. & Date	0.0
Scope	<p>This method is applicable for the determination of moisture in dried milk products such as whole, skimmed, partially skimmed milk powder, cream powder, infant milk food, infant formula, milk cereal weaningf, processed cereal based weaning food, milk protein concentrates, dairy permeates, colostrum powder, dairy whitener, dried ice-cream mix.</p> <p>This method gives free moisture content of the powder, i.e. it does not include water of crystallization of alpha lactose monohydrate. The total moisture, including water of crystallization can be determined by Karl Fischer titration or toluene distillation. However, sample of commerce are expected to contain less than 5% of free moisture, therefore oven method at 100 ± 2 °C is adequate for its determination</p>		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The sample is dried to constant weight at 102 ± 2 °C and the loss in weight reported as moisture.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Flat-bottom moisture dishes with covers made of stainless steel, nickel or aluminium having approximately 50 mm diameter and 25 mm depth. The dishes shall have lids which fit well and can readily be removed. 2. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Analytical balance (Readability 0.0001 g). 		
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Uncover a dish and place the dish and its lid in a hot air oven at 102 ± 2 °C for 1 h. Place the lid on the dish, transfer the covered dish from the hot air oven to the desiccator; allow it to cool to 25 ± 3 °C and weigh. 2. Place approximately 1 g of the the test powder in the dish, cover the dish with the lid and weigh the covered dish accurately and quickly. 3. Uncover the dish and place it along with its lid in the hot air oven maintained at 102 ± 2 °C for 2 h. 		

	<ol style="list-style-type: none"> 4. Replace the lid, transfer the covered dish to the desiccator, allow it to cool to 25 ± 3 °C (for approximately 30-45 min) and weigh it accurately and quickly. 5. Heat the uncovered dish and lid in the hot air oven at 102 ± 2 °C for further 1 h; replace the lid, allow the covered dish to cool to room temperature (25 ± 3 °C) in the desiccator and weigh it. 6. Repeat the process of drying, cooling and weighing until the successive weighing do not differ by more than 0.5 mg. It is usually found that drying is complete after the first 2 h.
Calculation with units of expression	$\text{Moisture \% by mass} = \frac{M_1 - M_2}{M_1 - M} \times 100$ <p>Where, M is mass in g, of the empty dish along with lid; M₁ is initial mass in g of the dish with lid and test portion; M₂ is the mass in g of the dish with lid and dried test portion</p> <p>The maximum deviation between duplicate determinations should not exceed 0.06% by mass of moisture.</p>
Reference	<ol style="list-style-type: none"> 1. IS 16072: 2012 (Reaffirmed year 2018), Determination of moisture content in milk powder and similar products, Bureau of Indian Standards: India. 2. ISO 5537/IDF 026:2004. Dried milk - Determination of moisture content (Reference method). International Organization for Standardization, Geneva. 3. F.A.O Manuals of Food quality Control 14/ 8 page 30.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Fat in Dried Milk Products by Reference Method		
Method No.	FSSAI 01.056:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in dried milk products such as whole, skimmed, partially skimmed milk powder, cream powder, infant milk food, infant formula, milk cereal weaning food, processed cereal based weaning food, milk protein concentrates, dairy permeates, colostrum powder, and dairy whitener.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	Sample is treated with ammonia and ethyl alcohol; the former to dissolve the protein and the latter to help precipitate the proteins. Fat is extracted with diethyl ether and petroleum ether. Mixed ethers are evaporated and the residue weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.		
Apparatus/Instruments	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021).		
Materials and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.		
Method of analysis	<p>Rose-Gottlieb Method</p> <ol style="list-style-type: none"> 1. Weigh 1 g of the sample in a Mojonnier flask or suitable extraction flask. 2. Add 10 mL of warm (65 ± 5 °C) distilled water. 		

	<p>3. Shake to dissolve the powder.</p> <p>4. Add 2 mL of ammonia solution, shake well and proceed as per the extraction fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021).</p>
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Reference	IS 11721: 2013 (Reaffirmed year 2018). Method of sampling and test for dried milk sample. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Fat in Whole Milk Powder by Gerber Method

Method No.	FSSAI 01.057:2021	Revision No. & Date	0.0
Scope	This method is applicable for the determination of fat in whole milk powder only.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Iso-amyl alcohol: It is a highly flammable liquid. Keep away from heat/sparks/open flames/hot surfaces. Perform all operations under a fume hood. 		
Principle	Sample is mixed with sulphuric acid and isoamyl alcohol in a special Gerber tube, permitting dissolution of the protein and release of fat. The tubes are centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the fat content of the milk powder sample.		
Apparatus/Instruments	<p>Refer apparatus requirement stated for milk (FSSAI 01.023:2021), along with following additional requirement.</p> <ol style="list-style-type: none"> 1. Analytical Balance (Readability 0.001 g). 2. Milk butyrometer of 10% scale (ISI marked). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Concentrated sulphuric acid (Approximately 36.8 N). 2. Isoamyl alcohol (furfural free). It should have density between 0.807 to 0.809 g/mL at 27 °C. 		
Preparation of Reagents	<p>Preparation of Gerber sulphuric acid: Take required volume of water in a pyrex flask (generally 100 mL of water is required for 900 mL of concentrated sulphuric acid) kept in a basin of ice-cold water. Carefully add the concentrated sulphuric acid in small quantities at a time keeping the container sufficiently cold and mix gently. After cooling the flasks, check the specific gravity of Gerber acid with hydrometer and if necessary, adjust the Gerber acid to the correct specific gravity with addition of water or acid, taking same precautions as before till specific gravity is in the range of 1.807 to 1.812 g/mL at 27 °C (or 1.815 to 1.820 g/mL at 20 °C). Store the prepared acid in a glass stoppered bottle to avoid</p>		

	absorption of water.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Mix the sample thoroughly, and weigh 1.69 ± 0.01 g of milk powder into the counterpoised scoop. 2. Add 10 mL of Gerber sulphuric acid into a butyrometer tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. 3. Add gently, from the wash bottle sufficient cold water to form a layer about 6 mm deep on top of the acid, allowing the water to flow down the side of the bulb. 4. Insert the narrow end of the stemless funnel into the neck of the butyrometer. Transfer the contents of the scoop to the funnel, removing the last particles with a camel hair brush. 5. Tap the funnel gently until most of the powder is in the butyrometer. Transfer the powder remaining in the funnel to the butyrometer with the aid of the glass rod and the camel hair brush. 6. Remove the funnel, add 1 mL of isoamyl alcohol, preferably by use of an automatic dispenser. 7. Add hot water ($70\text{ }^{\circ}\text{C}$) from the wash bottle until the butyrometer is filled to about 5 mm below the shoulder, allowing all air entrained in the powder to escape. 8. Close the neck of the butyrometer firmly with the stopper without disturbing the contents. 9. When a double ended stopper is used, screw it in until the widest part is, at least, level with the top of the neck. 10. When a lock stopper is used, insert it until the rim is in contact with the neck of the butyrometer. 11. Keep in a water bath at $65 \pm 2\text{ }^{\circ}\text{C}$ for at least 3 min and not less than 10 min, taking care to have casein particles, if any, to dissolve fully, and centrifuge for 5 min at 1100 rpm (approximately 300 g). 12. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. 13. Allow the centrifuge to come to rest. Remove the butyrometer tubes and place in water bath at $65 \pm 2\text{ }^{\circ}\text{C}$ for at least 3 min and not less than 10 min. 14. If after centrifuging, there is not a sharp dividing line between the fat and the acid, or if the acid layer is not clear, repeat the temperature adjustment and centrifuging before taking the reading. 15. Read the scale reading of the butyrometer

Calculation with units of expression	Calculation of Percentage & Fat - Multiply the scale reading of the butyrometer by the factor 20/3 to obtain the percentage of fat in milk powders.
Reference	IS 1224 (Part II): 1977 (Reaffirmed Year 2018). Determination of fat by the Gerber method: Milk products. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Titratable Acidity in Dried Milk Products

Method No.	FSSAI 01.058:2021	Revision No. & Date	0.0
Scope	This method is for the determination of titratable acidity in milk dried milk products such as whole, skimmed, partially skimmed milk Powder etc.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: It is extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	<p>In this method, reconstituted milk is prepared by addition of water to a test portion of dried milk, corresponding accurately to 5 g of solids-not-fat. It is titrated with 0.1 N sodium hydroxide solution using phenolphthalein as an indicator and cobalt (II) sulphate as reference colour solution. The number of milliliters of 0.1 N sodium hydroxide used in the titration is multiplied by the factor 2, in order to obtain the number of milliliters in terms of 10 g of solids-not-fat. The amount of sodium hydroxide solution required is a function of the amount of natural buffering substances present in the product and of developed or added acid or alkaline substances.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Burette, 50 mL graduated in 0.1 mL and with an accuracy of 0.05 mL. 3. Pipettes of 2 mL capacity. 4. Measuring cylinders of 50 mL capacity. 5. Conical flasks capacity 100- or 150-mL capacity with ground necks and ground glass stoppers. 		
Materials and reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Phenolphthalein. 3. Cobalt sulphate heptahydrate. 4. Ethanol. 		
Preparation of Reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide standard volumetric solution, (NaOH) = 0.1 ± 0.0002 N: Dissolve 0.4 g of sodium hydroxide in water and make up to 100 mL. Standardize the prepared solution with 0.1N standard 		

	<p>oxalic acid solution using phenolphthalein as indicator.</p> <ol style="list-style-type: none"> Reference colour solution: Dissolve 3 g of cobalt (II) sulphate heptahydrate (CoSO₄.7H₂O) in water and make up to 100 mL. Phenolphthalein solution: Dissolve 2 g of phenolphthalein in 75 mL of 95% (v/v) ethanol and add 20 mL of water. Add the sodium hydroxide solution until 1 drop gives a faint pink coloration, and make up to 100 mL of water.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> Determine the solids-not-fat content of the sample. For this, estimate the moisture (Refer method FSSAI 01.055:2021) and fat content (Refer method FSSAI 01.056:2021). Then, calculate the solids-not-fat by following equation $\text{Solids-not-fat (\%)} = 100 - \text{fat \%} - \text{moisture \%}$ Weigh (500/a) ± 0.01 g of the test sample into each of two conical flasks, “a” being the solids-not-fat content of the sample, expressed as a percentage to two decimal places. Prepare reconstituted milk by adding 50 mL of water at about 20 °C to the test portion and agitating vigorously. Allow to stand for about 20 min. Add to one of the conical flasks 2 mL of the reference colour solution to obtain a colour standard and mix by slight swirling. If a series of determinations on similar products is to be carried out, this colour standard may be used throughout. However, it should be discarded after 2 h. Add 2 mL of the phenolphthalein solution to the second conical flask, and mix by slight swirling. Titrate the contents of the second conical flask, while swirling, by adding the sodium hydroxide solution from the burette until a faint pink colour similar to that of the colour standard persists for about 5 sec. The titration should be completed within 45 sec. Record the volume, in millilitres, of sodium hydroxide solution used, to the nearest 0.05 mL.
Calculation with units of expression	<p>The titratable acidity = 2 x V</p> <p>Where, V = volume, in millilitres, of the sodium hydroxide solution used for titration</p>
Reference	IS 11766: 1986 (Reaffirmed Year 2018). Method for determination of titratable acidity in milk powder and similar products (routine method). Bureau of Indian Standards, New Delhi
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Titratable Acidity in Dairy Whitener

Method No.	FSSAI 01.059:2021	Revision No. & Date	0.0
Scope	This method is for the determination of titratable acidity in dairy whitener.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: It is extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	In this method, the dried test portion of dairy whitener is reconstituted with water and titrated with 0.1 N sodium hydroxide solution using phenolphthalein as an indicator and rosaniline acetate solution as reference colour solution. The amount of sodium hydroxide solution required is a function of the amount of natural buffering substances present in the product and of developed or added acid or alkaline substances.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Burette (50 mL), graduated in 0.1 mL and with an accuracy of 0.05 mL. 3. Pipettes of 2 mL capacity. 4. Measuring cylinders of 50 mL capacity. 5. Conical flasks capacity 100 or 150 mL capacity with ground necks and ground glass stoppers. 		
Materials and reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Phenolphthalein. 3. Rosaniline acetate. 		
Preparation of Reagents	<ol style="list-style-type: none"> 1. 0.1 N standard sodium hydroxide solution: Dissolve 0.4 g of sodium hydroxide in water and make upto 100 mL. Standardize the prepared solution with 0.1N standard oxalic acid solution using phenolphthalein as indicator. 2. Phenolphthalein Indicator solution 3. Rosaniline acetate solution (Stock solution) — Dissolve 0.12 g of rosaniline acetate in approximately 50 mL of rectified spirit [see IS: 		

	323-1959 Specification for Rectified Spirit (Revised)] containing 0.5 mL of glacial acetic acid. Make up to 100 mL with rectified spirit. 4. Rosaniline acetate solution (Bench Solution) — Dilute 1 mL of the stock solution to 500 mL with a mixture of rectified spirit (see IS: 323-1959) and distilled water in equal proportions by volume.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 1 g of the sample into each of the two porcelain dishes. 2. Add 10 mL of boiling water to each dish and stir with the flat end of a glass rod until a perfectly smooth liquid is obtained. 3. Cool to 25 ± 3 °C. Use the contents of one dish as a blank by stirring on 2 mL of bench solution of rosaniline acetate. 4. Add 1 mL of phenolphthalein indicator solution to the other dish followed by standard sodium hydroxide solution drop by drop from burette until by comparison the color matches the pink tint of the blank. 5. Stir vigorously throughout. The time taken for the complete titration shall not exceed 20 seconds. The titration shall be preferably made in north light or under illumination from a day light lamp.
Calculation with units of expression	<p>Titrate acidity (as % lactic acid) by mass = $\frac{9 AN}{M}$</p> <p>Where,</p> <p>A = volume in mL of the standard sodium hydroxide required for titration N = normality of the standard sodium hydroxide solution, and M = mass in g of milk powder taken for the test</p>
Reference	IS 1165: 2002 (Reaffirmed Year 2013). Milk Powder-Specifications. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Added Sugar in Dairy Whitener

Method No.	FSSAI 01.060:2021	Revision No. & Date	0.0
Scope	This method is for the determination of added sugar in dairy whitener.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 		
Principle	<p>The principle involves clarifying the sample with the help of zinc acetate and potassium ferrocyanide, inverting a part of the sample using hydrochloric acid and determining the reducing sugars content in both inverted and non-inverted parts of the sample. The sugar content is estimated by determining the volume of the unknown sugar solution required to completely reduce a measured volume of Fehling's solution. Invert sugar reduces the copper in Fehling's solution to red insoluble cuprous oxide.</p>		
Material and Reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Sucrose. 3. Methylene blue. 4. Copper sulphate (CuSO₄.5H₂O). 5. Concentrated sulphuric acid (Approximately 36.8 N). 6. Rochelle's salt (potassium sodium tartrate –KNaC₄H₄O₆.4H₂O). 7. Zinc acetate. 8. Potassium ferrocyanide. 9. Concentrated hydrochloric acid (Approximately 11.6 N). 		


	<p>10. Concentrated ammonia (Specific gravity 0.91).</p> <p>11. Acetic acid.</p>
Preparation of reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide solution: Approximately 0.1 N prepared from sodium hydroxide, analytical reagent grade. 2. Stock solution of invert sugar: Weigh accurately 9.5 g of pure sucrose and transfer it to a one litre volumetric flask with 100 mL of water. Add 5mL of concentrated hydrochloric acid. Allow this to stand for 3 days at 20 to 25 °C and then make up to volume with water (This solution is stable for several months). 3. Standard solution of invert sugar: Neutralize 40 mL of the stock solution of invert sugar (Reagent B) with sodium hydroxide solution using litmus paper and dilute with water to 100mL volume. 4. Methylene blue indicator solution: Dissolve 0.2 g of methylene blue in water and dilute to 100 mL. 5. Fehling's solution (Soxhlet modification): Prepared by mixing immediately before use, equal volumes of Solution A and Solution B, prepared as described below. <ol style="list-style-type: none"> a) Solution A: Dissolve 34.639 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water add 0.5 mL of concentrated sulphuric acid and dilute to 500 mL in a volumetric flask. Filter the solution through prepared asbestos. b) Solution B: Dissolve 173 g of Rochelle salt and 50 g of sodium hydroxide analytical reagent grade in water, dilute to 500 mL in a volumetric flask and allow the solution to stand for two days, Filter this solution through prepared asbestos. 6. Standardization of Fehling's solution: Pipette accurately 10 mL of each Fehling's solutions (solution A and B) into a 250 mL Erlenmeyer flask. Pour the standard solution of invert sugar in burette and run in from the burette almost whole of the invert sugar solution required to reduce all of the copper so that not more than 1 mL is required to complete the titration. Heat the contents on wire gauze. Gently boil the contents for 2 min. Add about 1 mL of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add standard invert sugar solution till the blue colour of the solution disappears. Titration should be completed within 1 min so that contents of the flask boil altogether for 3 min without interruption. 7. Zinc acetate solution: Dissolve 21.9 g of crystalline zinc acetate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$) in water and add 3 mL of glacial acetic acid. Make up to 100 mL.

	8. Potassium ferrocyanide solution: Dissolve 10.6 g of crystalline potassium ferrocyanide and make up to 100 mL with water. 9. Dilute ammonia solution: 10 mL of concentrated ammonia solution diluted to 100 mL with water. 10. Dilute acetic acid solution: Approximately equivalent to the dilute ammonia solution in strength.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation
Method of analysis	1. Weigh accurately about 40 g of the well - mixed sample and transfer to 100 mL beaker. Add 50 mL of hot water at 80 to 90 °C. Mix and transfer to a 250 mL measuring flask washing it with successive quantities of distilled water at 60 °C, until the volume is 120 to 150 mL. 2. Mix and cool to room temperature (25 ± 3 °C) and add 5 mL of the dilute ammonia solution. Mix and allow to stand for 15 min. 3. Add the exact equivalent of dilute acetic acid to neutralize the ammonia added. Mix and add 12.5 mL of zinc acetate solution followed by 12.5 mL of potassium ferrocyanide solution. 4. Mix again. Make up to 250 mL mark. Allow to settle and filter. Mark this solution B-I. 5. Pipette 50 mL of solution B-I into a 100 mL volumetric flask, add 5 mL of concentrated hydrochloric acid and heat in a water bath at 65 – 68 °C for 5 min rotating the flask for the first 3 min. 6. Cool the solution and neutralize with sodium hydroxide solution. Mark this solution A- I. Make up to 100 mL. 7. Dilute the solutions B-I (usually 50 mL of B-I solution is required to be diluted to 100 mL to obtain titration reading of around 14 mL when titrated against Fehling solution) and A-I (usually 15 mL of A-I solution is required to be diluted to 100 mL to obtain titration reading of around 18 mL when titrated against Fehling solution) so that the volume of solution required to react with 10 mL Fehling's solution is between 15 and 50 mL. 8. Mark them B-II and A-II, respectively. Standard method of titration 1. Pour the solution (BII) in a 50 mL burette. Pipette 5 mL of Fehling solution A and 5 mL of Fehling solution B into a 250 mL conical flask and run into the solution from the burette to effect reduction of all the copper (blue colour faints) 2. Boil the contents of flask for 2 min. At the end of 2 min of boiling add, without interrupting boiling one mL of methylene blue indicator solution. While the contents of the flask continue to boil, add the

	<p>prepared solution from the burette until the blue colour disappears. The titration should be completed within 3 min without interruption.</p> <p>3. Repeat the steps of standard method of titration using solution AII.</p>
Calculation with units of expression	<p>Sucrose, percent by weight = $\frac{25*W1}{W2} \left[\frac{2f2}{V2} - \frac{f1}{V1} \right]$</p> <p>Where,</p> <p>W1= weight in mg of sucrose corresponding to 10 mL of Fehling's solution</p> <p>W2 = weight in g of the material taken for the determination</p> <p>f2 = dilution factor for solution A - II from A - I</p> <p>V2 = volume in mL of solution A - II corresponding to 10 mL of Fehling's solution</p> <p>f1 = dilution factor for the solution B - II from B - I</p> <p>V1 = volume in mL of solution B - II corresponding to 10 mL of Fehling's solution</p>
Reference	Appendix C of IS 4079: 1967 (Reaffirmed year 2016). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Milk Protein (Milk Solids-Not-Fat Basis) in Dried Milk Products		
Method No.	FSSAI 01.061:2021	Revision No. & Date	0.0
Scope	This scope is for the determination of protein content in dried milk products such as whole, skimmed, partially skimmed milk powder, cream powder and dairy whitener.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk (FSSAI.01.025/026:2021). To calculate the protein on solids-not-fat basis, milk fat and moisture are calculated separately and finally the results are presented on the basis of % milk protein in milk solids-not-fat.		
Apparatus/Instruments			
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.		

Method of analysis	<p>1. Weigh quickly about 0.5 g of the prepared dahi/yoghurt sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube.</p> <p>2. Refer the method for determination of protein in milk and milk products.</p> <p>Determination of fat Refer method for determination of fat in dried powder (FSSAI 01.056/057:2021)</p> <p>Determination of moisture Refer method for determination of moisture in dried powder (FSSAI 01.055:2021)</p>
Calculation with units of expression	<p>Refer the method for determination of protein in milk and milk products (FSSAI 01.026:2021)</p> <p>For milk powder Total Solids (%) = 100 - moisture (%) Milk Solids not fat = Total Solids (%) – Fat (%)</p> <p>For Dairy Whitener Total Solids (%) = 100 - moisture (%) Milk Solids not fat (%) = Total Solids (%) – Sugar (%) - Fat (%)</p> $\text{Protein on milk SNF basis} = \frac{\% \text{ Protein} \times 100}{\% \text{ Milk SNF}}$
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Milk Protein in Foods for Infant Nutrition and Processed Cereal Based Complementary Foods		
Method No.	FSSAI 01.062:2021	Revision No. & Date	0.0
Scope	This scope is for determination of milk protein in infant milk food, infant formula, milk cereal weaning food, processed cereal based weaning food, milk protein concentrates, dairy permeates and colostrum powder.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information</p> <p>Safety of</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk and milk products (FSSAI 01.026:2021)		
Apparatus/Instruments			
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.		

Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 0.5 g of the prepared sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube. 2. Refer the method for determination of protein by Kjeldahl in milk and milk products (FSSAI 01.025/026:2021).
Calculation with units of expression	Refer the method for determination of protein by Kjeldahl in milk and milk products (FSSAI 01.025/026:2021).
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Total Ash in Dried Milk Products


Method No.	FSSAI 01.063:2021	Revision No. & Date	0.0
Scope	This method is for the determination of total ash on dry basis in dried milk products such as whole, skimmed, partially skimmed milk powder, cream powder, infant milk food, infant formula, milk cereal weaning food, processed cereal based weaning food, milk protein concentrates, dairy permeates, colostrum powder, and dairy whitener. This method is also for the determination of total ash on fat free, moisture free and sugar free basis.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot crucibles. 2. Hot crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	Destruction of organic matter by incinerating the sample to a constant mass at high temperature of $(550 \pm 20 \text{ }^\circ\text{C})$ in Muffle furnace.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Platinum or silica crucible, about 70 mm diameter and 25 to 50 mm deep. 2. Muffle furnace, capable of being controlled at $550 \pm 20 \text{ }^\circ\text{C}$. 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Safety tongs having long arms. 5. Bunsen burner or electric hot plate. 6. Analytical balance (Readability 0.0001g) 7. Thermal protection gloves, capable to resist temperature up to $550\text{-}600 \text{ }^\circ\text{C}$ 8. Hot air oven (forced air type), capable of being controlled at $102 \pm 2 \text{ }^\circ\text{C}$ 		
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 3 g of the dried milk sample in a crucible, previously dried in a hot air oven and weighed. 2. Heat the crucible gently on a burner or hot plate at first and then strongly in a muffle furnace at $550 \pm 20 \text{ }^\circ\text{C}$ till grey ash is obtained. 3. Cool the crucible in a desiccator and weigh it. 4. Heat the crucible again at $550 \pm 20 \text{ }^\circ\text{C}$ for 30 min. 5. Cool the crucible in a desiccator and weigh. 6. Repeat this process of heating for 30 min, cooling and weighing until the difference between two successive weighing is less than 1 mg. 		

	<p>7. Record the lowest mass.</p> <p>Determination of fat Refer method for determination of fat in dried powder (FSSAI 01.056/057:2021)</p> <p>Determination of moisture Refer method for determination of moisture in dried powder (FSSAI 01.055:2021)</p> <p>Determination of added sugar (In Dairy Whitener) Refer method for determination of added sugar in dried powder (FSSAI 01.060:2021)</p>
Calculation with units of expression	<p>Total ash % by mass = $\frac{M_2 - M}{M_1 - M} \times 100$</p> <p>Where, M_2 = mass in g, of the crucible with ash M = mass in g, of the empty crucible M_1 = mass in g, of the crucible with the material taken for the test and</p> <p>Total ash (% on dry matter basis) = $\frac{\text{Total ash (\%)}}{\text{Total ash (\%)}} \times 100$</p> <p>Total ash (% on fat and moisture free basis) = $\frac{\text{Total ash (\%)}}{(100 - \text{Moisture (\%)} - \text{fat (\%)})} \times 100$</p> <p>Total ash (% on fat, sugar and moisture free basis) = $\frac{\text{Total ash (\%)}}{(100 - \text{Moisture (\%)} - \text{fat (\%)} - \text{sugar (\%)})} \times 100$</p>
Reference	IS 1165: 2002 (Reaffirmed year 2018). Milk Powder – Specifications. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Acid Insoluble Ash in Dried Milk Products

Method No.	FSSAI 01.064:2021	Revision No. & Date	0.0
Scope	This method is for the determination of ash insoluble in hydrochloric acid (for infant foods and processed cereal based weaning foods and Dairy Whitener).		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Silver nitrate: It is corrosive. It is not combustible, but it is a strong oxidizer that enhances the combustion of other substances. <p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot crucibles. 2. Hot crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The 'Acid Insoluble Ash' content is the proportion of the ash that is not soluble in acid and is not subsequently volatilised upon the incineration of this acid insoluble residue.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Platinum or silica crucible about 70 mm diameter and 25 to 50 mm deep. 3. Muffle furnace, capable of being controlled at 550 ± 20 °C. 4. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 5. Safety tongs having long arms. 6. Bunsen burner or electric hot plate. 7. Boiling water bath (100 ± 2 °C) 8. Ashless Whatman filter paper Grade 42. 9. Watch-glass. 		

	10. Hot air oven (forced air type), capable of being controlled at 100 ± 2 °C.
Materials and reagents	1. Concentrated hydrochloric acid (Approximately 11.6 N). 2. Silver nitrate.
Preparation of reagents	1. Dilute hydrochloric acid (5 N): Dilute 43 mL of concentrated hydrochloric acid to 100 mL with distilled water. 2. Silver nitrate solution: 0.1 N: Dissolve accurately 1.6987 g of silver nitrate in 25 mL distilled water and makeup the volume to 100 mL with distilled water.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.
Method of analysis	1. To the ash contained in the crucible (obtained in the previous method), add 25 mL of dilute hydrochloric acid (5 N), cover it with a watch-glass and heat on water-bath for 10 min. 2. Allow to cool and filter the contents of the crucible through an ashless Whatman filter paper Grade 42. 3. Wash the filter paper with water until the washings are free from the acid [check with AgNO ₃ solution (0.1 N solution); till the washings do not form white precipitate with AgNO ₃ solution] and return the washed filter paper to the crucible. 4. Keep the filter paper in a hot air oven maintained at 100 ± 2 °C for about 3 h. 5. Ignite the crucible in a muffle furnace at 550 ± 20 °C for 1 h. Cool the crucible in a desiccator to room temperature (for approximately 30-45 min) and weigh it. 6. Heat the crucible again at 550 ± 20 °C for 30 min, cool in a desiccator and weigh. Repeat this process of heating for 30 min, cooling and weighing until the difference between two successive weighing is less than 1 mg. 7. Record the lowest mass.
Calculation with units of expression	$\text{Acid insoluble ash, \% (m/m)} = \frac{M_2 - M}{M_1 - M} \times 100$ <p>Where, M is mass in g of the empty crucible; M₁ is mass in g of the crucible with the material taken for the test; M₂ is mass in g of the crucible with acid insoluble ash</p>
Reference	IS 1165 – 2002 (Reaffirmed year 2018). Milk Powder – Specifications. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Crude Fibre Content in Milk Cereal Based Complementary Food and Processed Cereal Based Complementary Foods		
Method No.	FSSAI 01.065:2021	Revision No. & Date	0.0
Scope	This method is for the determination of crude fibre content in milk cereal based complementary food and processed cereal based complementary foods.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Petroleum ether: Extremely flammable. Avoid breathing fumes, perform all operations under a fume hood. Keep away from heat, sparks and open flame. 		
Principle	In this method, after any necessary grinding and defatting, the sample is boiled with sulphuric acid solution of standard concentration, the insoluble residue is then separated and washed. The obtained residue is then boiled with sodium hydroxide solution of standard concentration; it is then separated, washed and dried. The residue is then weighed and the loss in mass on incineration is determined.		


Apparatus/Instruments	<ol style="list-style-type: none"> 1. Grinding device which is easy to clean, suited to the nature of the product and allows grinding of the product without causing undue heating or significant change in the moisture content. 2. Burette graduated in 0.1 mL and with an accuracy of 0.05 mL. 3. Sieve, of metal wire cloth, aperture size 1 mm, complying with the requirements of ISO 3310/ 1. 4. Hot air oven (forced air type), capable of being controlled at 130 ± 2 °C. 5. Wide-mouthed vessel, provided with a condenser, for example a flask having a minimum capacity of 600 mL fitted with a reflux condenser, or a beaker without spout, of capacity 600 mL, covered by a round-bottom flask of 500 mL capacity containing 456 mL of cold water or automatic system. 6. Heating device, for example an electrically heated hotplate fitted with a magnetic stirrer, capable of maintaining 200 mL of reagents at a gentle boil. 7. Incineration dish of capacity 25 to 50 mL, resistant to attack under the test conditions, or a filter crucible, suitable for separation and incineration of the residue. 8. Muffle furnace, provided with air circulation and temperature control, suitable for carrying out incineration at 550 ± 25 °C. 9. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 10. Separating device. 11. Analytical balance (Readability 0.0001 g).
Materials and reagents	<ol style="list-style-type: none"> 1. Concentrated sulphuric acid (Approximately 36.8 N). 2. Sodium hydroxide. 3. Acetone or 95% ethanol, methanol or propan-2-ol. 4. Concentrated hydrochloric acid (Approximately 11.6 N). 5. n-hexane, petroleum ether, diethyl ether or any other solvent.
Preparation of Reagents	<ol style="list-style-type: none"> 1. Sulphuric acid solution: Standard volumetric solution 0.255 ± 0.005 N. Take 12.68 mL of concentrated sulphuric acid and make up to the volume to 1000 mL in a volumetric flask. 2. Sodium hydroxide solution: Standard volumetric solution, (0.313 ± 0.005 M). Weigh 12.5 g of sodium hydroxide. Dissolve in water and make up to 1000 mL This solution shall be as free as possible from carbonates. 3. Acetone or 95% (v/v) ethanol, or methanol, or propan-2-ol. 4. Extraction solvent: Technical grade n-hexane, or light petroleum (having a boiling range between 40 and 60 °C), or diethyl ether, or

	<p>another solvent or mixture of solvents more suitable for the extraction of fatty substances from certain products to be analysed.</p> <ol style="list-style-type: none"> 5. Hydrochloric acid: 0.5 mol/L solution (in the case of products rich in carbonates). Carefully add 21.6 mL of concentrated hydrochloric acid to water in a standard flask. Make up the volume to 500 mL with distilled deionised water. 6. Filter aid. 7. Anti-foam agent (for example an aqueous emulsion containing 30 percent m/m of silicone), if necessary, known to have no effect on the results. 8. Anti-bumping agent (glass beads), if necessary, resistant to attack under the test conditions or known to have no effect on the results.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.
Method of analysis:	<p>Products requiring grinding: Products that do not pass through the test sieve without leaving a residue, shall be ground. If the results are to be expressed relative to the product as received, determine beforehand the dry matter content of the sample by an appropriate method. Grind the laboratory sample in the grinding device so that the product passes through the sieve without leaving a residue.</p> <p>Extraction of fatty substances: If the fatty matter content is less than 1%, extraction of fatty matter is not required. Extraction is recommended if the fat content is between 1 and 10%. If the fatty matter content is more than 10%, prior extraction is essential. For products containing fatty substances which cannot be removed directly, the extraction shall be carried out after the acid treatment.</p> <ol style="list-style-type: none"> 1. Weigh to the nearest 1 mg, about 3 g of the test sample and which is presumed to contain more than 1% of crude fibre. 2. In the case of products having moisture content too high for them to be mixed or ground as received, carry out a preliminary drying of the product at an appropriate temperature. 3. In this case, weigh the product before the preliminary drying and again just before preparation of the test sample. <p>Acid treatment:</p> <ol style="list-style-type: none"> 1. Transfer the test portion, which may have had the fat and oils and carbonates removed into the vessel. 2. Add the prescribed quantity of filter aid and the anti-foam agent and the anti-bumping agent. 3. Measure 200 mL of the sulphuric acid solution, bring it to a temperature of 95 to 100 °C and add it to the contents of the vessel. 4. Fit the condenser, bring it rapidly to the boil (in about 2 min) using

	<p>the heating device and continue boiling gently for 30 ± 1 min.</p> <ol style="list-style-type: none"> 5. Swirl the vessel from time to time so that any particles adhering to the interior walls are returned to the solution. 6. After the specified boiling period, add about 50 mL of cold water and separate rapidly the insoluble residue using the separating device chosen. 7. Wash the vessel with 50 mL portions of hot water (of temperature 95 to 100 °C) and pour the washings over the insoluble residue remaining in the separating device. 8. Repeat the washing of the insoluble residue until the filtrate is substantially neutral to litmus paper. 9. The separation and washing of the insoluble residue shall be completed in less than 30 min. <p>Alkaline treatment:</p> <ol style="list-style-type: none"> 1. Return the washed insoluble residue to the vessel and add the anti-foam agent and the anti-bumping agent. 2. Measure 200 mL of the sodium hydroxide solution, bring it to a temperature of 95 to 100 °C. 3. Fit the condenser and bring rapidly to boil (in about 2 min) using the heating device, and continue to boil gently for 30 ± 1 min. 4. After the specified boiling period, add about 50 mL of cold water and rapidly separate the insoluble residue using the separating device chosen. 5. Wash the residue with 25 mL of the sulphuric acid solution measured at room temperature (25 ± 3 °C) and then raised to a temperature of 95 to 100 °C. 6. Wash with water as described in Dry the residue with one of the reagents; wash with solvent to remove unsaponifiable fatty matter. According to the filtration technique chosen, collect the entire residue in the incineration dish or in the filter crucible. <p>Drying:</p> <ol style="list-style-type: none"> 1. Dry the incineration dish or the filter crucible with its contents in the oven at 130 ± 2 °C. 2. Allow to cool to room temperature (25 ± 3 °C) in the desiccator and quickly weigh to the nearest 0.5 mg. 3. Repeat these operations until the difference between two successive weighing, following drying in the oven and cooling in the desiccator, does not exceed 1 mg. <p>Incineration:</p> <ol style="list-style-type: none"> 1. After drying, incinerate the dry residue in the muffle furnace at 550
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	<p>± 25 °C to constant mass.</p> <p>2. Allow to cool to room temperature (25 ± 3 °C) in the desiccator and weigh again to the nearest 0.5 mg.</p> <p>Number of determinations: Carry out at least two determinations on the same test sample.</p> <p>Blank test: If asbestos is used as a filter aid, carry out a blank test under the same conditions as described in.</p>
<p>Calculation with units of expression</p>	<p>Crude fibre content relative to product as received: The crude fibre content, expressed as percentage by mass relative to the product as received, is given by the following formula:</p> <p>a) for products not requiring grinding:</p> $\text{Crude fibre} = [M_1 - (M_2 + M_3)] \times \frac{100}{M_0}$ <p>b) for products requiring grinding:</p> $\text{Crude fibre} = [M_1 - (M_2 + M_3)] \times \frac{100}{M_0} \times \frac{100}{M''s} \times \frac{Ms}{100}$ <p>Where, M₀ is mass, in g, of the test portion M₁ is total mass, in g, of the dry residue and its support after drying, M₂ is total mass, in g, of the dry residue and its support after incineration M₃ is difference in mass, in g, observed during the incineration process in the blank test, taking into account the quantity of filter aid used; M_s is dry matter content, expressed as a percentage by mass, of the product as received, M''_s is dry matter content, expressed as a percentage by mass, of the test sample,</p> <p>Crude fibre content relative to the dry matter content of the product: The crude fibre content, expressed as a percentage by mass relative to the dry matter content of the product, is given by the following formulae:</p> $\text{Crude fiber} = [M_1 - (M_2 + M_3)] \times \frac{100}{M_0} \times \frac{100}{M''s}$ <p>Where, M₀ is the mass, in g, of the test portion M₁ is the total mass, in g, of the dry residue and its support after drying, M₂ is the total mass, in g, of the dry residue and its support after incineration, M₃: is the difference in mass, in grams, observed during the incineration</p>

	<p>process in the blank test, taking into account the quantity of filter aid used; M's: is the dry matter content, expressed as a percentage by mass, of the test sample,</p> <p>Case of preliminary drying:</p> <p>If a preliminary drying was carried out, the crude fibre content, expressed as a percentage by mass relative to the product as received, is obtained by multiplying the result calculated according to crude fibre content relative to the product as received by the ratio:</p> M_5/M_4 <p>Where,</p> <p>M₄: is the mass, in g, of the initial moist sample before preliminary drying, M₅: is the mass, in g, of the same sample after the preliminary drying.</p>
Reference	IS 10226 (Part 1): 1982 (Reaffirmed Year 2015). Method for determination of crude fibre content: Part 1 General method. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

		Determination of Total Carbohydrates in Milk Cereal Based Complementary Food and Processed Cereal Based Complementary Food	
Method No.	FSSAI 01.066:2021	Revision No. & Date	0.0
Scope	This method is for the determination of total carbohydrates in milk cereal based complementary food and processed cereal based complementary food.		
Caution	This method is based on calculation and thus follow the caution listed in individual method of determination (moisture, fat, protein and ash content).		
Principle	Total carbohydrate is determined by calculation method. After adding moisture, fat, protein and ash content; the value obtained is then deducted from 100.		
Apparatus/Instruments	This method is based on calculation and thus use the apparatus/instruments listed in individual method of determination (moisture, fat, protein and ash content).		
Material and Reagents	This method is based on calculation and thus use the materials and reagents listed in individual method of determination (moisture, fat, protein and ash content).		
Preparation of reagents	This method is based on calculation and thus use the preparation of reagents listed in individual method of determination (moisture, fat, protein and ash content).		
Sample preparation	This method is based on calculation and thus use the sample preparation listed in individual method of determination (moisture, fat, protein and ash content).		
Method of analysis	1. Total carbohydrate is determined by subtracting from 100, the addition of moisture (FSSAI 01.055:2021), fat (FSSAI 01.056/057:2021), protein (FSSAI 01.061:2021) and ash content (FSSAI 01.063:2021).		
Calculation with units of expression	Total carbohydrate including sucrose, dextrose and dextrin, maltose or lactose percent by weight = $100 - (A+B+C+D)$ Where A = Percent by mass of moisture B = Percent by mass of total protein C = Percent by mass of fat and D = Percent by mass of total ash		
Reference	IS 1656: 2017 (Reaffirmed year 2018). Milk cereal based Weaning foods – Specification. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

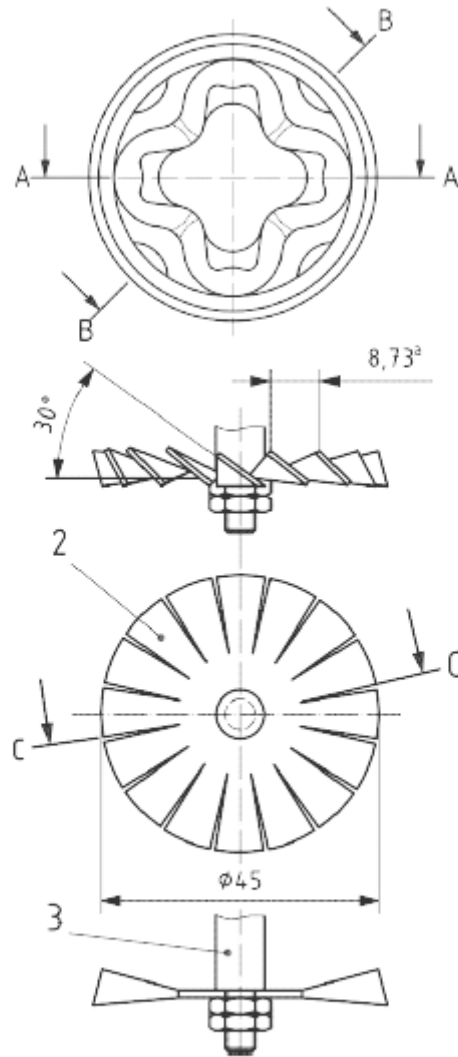
Determination of Insolubility Index of Dried Milk Products

Method No.	FSSAI 01.067:2021	Revision No. & Date	0.0
Scope	This method is for the determination of insolubility index in dried milk products such as whole, skimmed, partially skimmed milk powder, milk protein concentrates and dairy whitener.		
Caution	Wear laboratory apron, shoes, safety goggles and mask while working in the laboratory.		
Principle	Water at 24 °C (or at 50 °C if appropriate) is added to a test portion, which is reconstituted using a special mixer. After a specified standing period, a specified volume of the reconstituted milk or milk product is centrifuged in a graduated tube. The supernatant liquid is removed and the sediment is re-dispersed after the addition of water at the same temperature as used for the reconstitution. The mixture is centrifuged and the volume of sediment (insoluble residue) obtained is recorded.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Thermometer, capable of measuring a temperature of 24 °C or 50 °C with an error not exceeding ± 0.2 °C. 2. Water bath, capable of being maintained at 24.0 ± 0.2 °C and/or $50 \text{ °C} \pm 0.2$ °C, in which one or more mixing jars can be placed. 3. Mixing jar made of glass, of capacity 500 mL, as supplied for use with the mixer. 4. Scoop, with a smooth surface, or sampling paper, black, glazed (of dimensions 140 mm \times 140 mm), for weighing the test portion. 5. Analytical balance (Readability 0.01 g). 6. Measuring cylinder made of plastic material, of capacity 100 mL \pm 0.5 mL. 7. Stainless steel brush, suitable for removing any residual test portion from the scoop or sampling paper. 8. Electric mixer, equivalent to that manufactured for the solubility index method of the American Dry Milk Institute (ADMI) with the following characteristics: <ol style="list-style-type: none"> a. The 16-bladed impeller (stainless steel) shall have the shape and diameter shown in Fig. 1 and shall be attached to the shaft of the mixer so that the “flat” side of the impeller is underneath, as also shown in Fig. 1. The slope of the blades is upward from right to left; this is for clockwise rotation (see the Note). b. The pitch of the impeller blades shall be 30° and the horizontal distance between the blades (around the circumference of the impeller) shall be 8.73 mm, as shown in Fig. 1. With usage of the 		

impeller, these dimensions can change and hence periodic inspection and maintenance are essential.

- c. When the mixing jar is fitted to the mixer, the length of the mixer shaft shall be such that the distance from the lowest part of the impeller to the bottom of the jar is $10 \text{ mm} \pm 2 \text{ mm}$; this means that for a jar of depth 132 mm the distance from the top of the jar to the lowest part of the impeller is $122 \text{ mm} \pm 2 \text{ mm}$, and to the plane of the lowest part of the impeller blades is $115 \text{ mm} \pm 2 \text{ mm}$. The impeller shall also be located centrally in the jar.
- d. When the mixing jar, containing 100 mL of water at $24 \text{ }^\circ\text{C}$ with or without the addition of an appropriate test portion is fitted to the mixer and the mixer switched on, the impeller shall come to its operational fixed rotational frequency of $(3600 \pm 100) \text{ min}^{-1}$ in less than 5 s. The direction of rotation of the impeller shall be clockwise (viewed from above). The rotational frequency of the impeller under load (as described above) shall be checked periodically with an electronic tachometer; this is especially necessary with older models of the mixer, with a non-synchronous motor, whose rotational frequency has to be adjusted to $(3600 \pm 100) \text{ min}^{-1}$ by means of a speed control and a speed indicator (which may not remain accurate).

NOTE: Some mixers for the ADMI method cause the impeller to rotate in an anticlockwise direction (viewed from above). These mixers require an impeller whose blades slope upward from left to right so that liquid in the mixing jar moves in the same way as with a clockwise rotating impeller. In all respects, including its manner of attachment to the shaft and its distance from the bottom of the mixing jar, an anticlockwise-rotating impeller is equivalent to a clockwise-rotating impeller.



Dimensions in millimetres

Key

- 1 mixer jar 2 impeller 3 mixer shaft
 a Horizontal distance between blades (around circumference)

Fig-1: Mixing Jar and Impeller (Source: IS 12789: 2019)

9. Interval timer, indicating 0 s to 60 s and 0 min to 60 min.
10. Spoon spatula of length approximately 210 mm.
11. Centrifuge tubes of glass, conical, with the shape, dimensions, graduation scheme, inscriptions and patch of matt surface shown in Fig. 2, and provided with rubber stoppers. The graduation lines, graduation numbers and the inscription “mL (20 °C)” shall be marked in a permanent manner and the graduation lines shall be fine and clean. The maximum errors (\pm) in capacity at 20 °C shall be as follows: at 0.1 mL: ± 0.05 mL; from 0.1 mL to 1 mL inclusive: ± 0.1 mL; from 1 mL to 2 mL inclusive: ± 0.2 mL; from

2 mL to 5 mL inclusive: ± 0.3 ; mL from 5 mL to 10 mL: ± 0.5 mL; at 10 mL: ± 1 mL. For routine production control purposes, tubes of different dimensions may be used provided that they comply with limits for maximum error in capacity, as listed above. In all cases of dispute or where a definitive result is required, the tubes specified in this sub clause should be used.

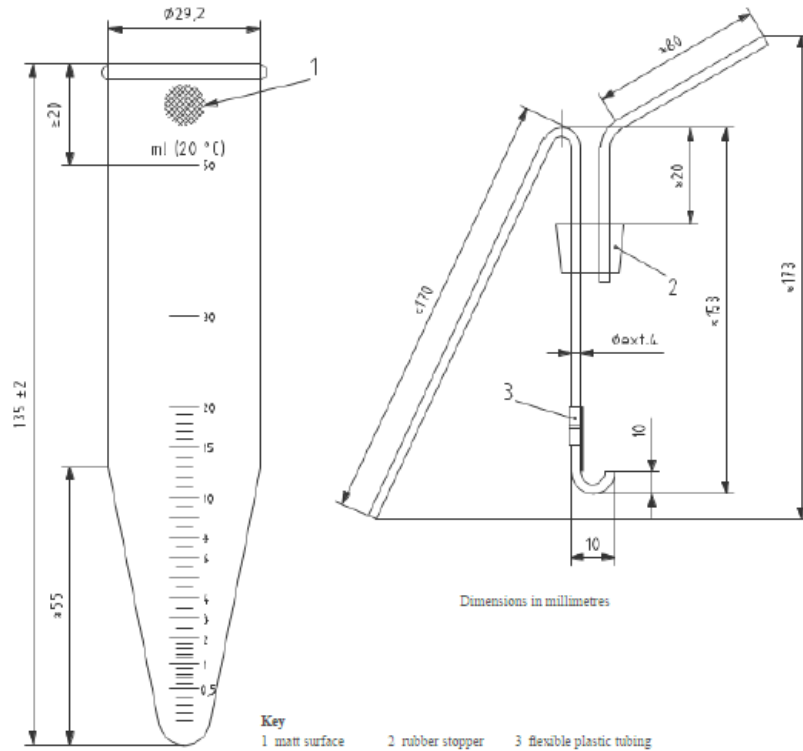


Fig-2: Centrifuge tube and Siphon Fitting (Source: IS 12789: 2019)


12. Centrifuge, electric, with speed indicator (min^{-1} or r/min), with vertical-loading swing-out cups for accommodating the centrifuge tubes and capable of producing an acceleration of $160 g_n$. At the internal bottom of the tubes and maintaining a temperature of 20°C to 25°C within the closed centrifuge.
 - NOTE — The acceleration, in terms of g_n , produced in a centrifuge is equal to

$$1.12 \text{ m}^2 \times 10^{-6}$$
 where, r = effective horizontal radius of spinning, in millimeters;
 n = rotational frequency, per min.
13. Siphon fitting or suction tube attached to water pump, made of glass tubing and with upturned tip, for removing supernatant liquid from a centrifuge tube

	<p>14. Stirring rod made of glass, of length 250 mm and diameter 3, 5 mm.</p> <p>15. Magnifying lens, suitable for aiding the reading of the volume of sediment.</p>
Materials and reagents	Silicone antifoaming agent.
Preparation of Reagents	Silicone Antifoaming Agent (for example an aqueous emulsion containing 30 percent m/m of silicone): Test the suitability of the silicone antifoaming agent by carrying out the procedure described in the method without a test portion. No more than a trace of silicone fluid (≤ 0.01 mL) should be visible at the bottom of the tube at the end of the procedure.
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. According to whether the insolubility index is to be measured at 24 °C or at 50 °C, adjust the temperature of the mixing jar to 24.0 °C \pm 0.2 °C or 50.0 °C \pm 0.2 °C, respectively, by keeping the jar in the water bath, with the water level near the top of the jar, for a sufficient period of time. Weigh, to the nearest 0.01 g, in the scoop or on the sampling paper, a test portion of: <ol style="list-style-type: none"> a) 13.00 g, in the case of dried whole milk, dried partly skimmed milk, and infant food based on either of these, b) 10.00 g, in the case of dried skimmed milk and dried buttermilk, or c) 7.00 g, in the case of dried whey. 2. Remove the mixing jar from the water bath, quickly wipe dry the outside of the jar and, using the measuring cylinder, add to the jar 100 mL \pm 0.5 mL of water at 24.0 °C \pm 0.2 °C or 50.0 °C \pm 0.2 °C as appropriate. 3. Add 3 drops of the silicone antifoaming agent to the water in the mixing jar and then transfer the test portion to the jar, using the brush, if necessary, so that the entire test portion falls onto the surface of the water. 4. Fit the mixing jar to the mixer, switch on the mixer, and after the mixer has operated for exactly 90 s, switch it off. 5. If the mixer is of the type with a nonsynchronous motor, speed control and speed indicator, bring the rotational frequency of the impeller to 3600 \pm 100 min⁻¹ within the first 5 secs of the 90 sec mixing period. 6. Remove the mixing jar from the mixer (allow a few secs for liquid on the impeller to drain into the jar) and leave the jar to stand at laboratory temperature for not less than 5 min and not more than 15 min. 7. Add 3 drops of the silicone antifoaming agent to the mixture in the

	<p>mixing jar; thoroughly mix the contents of the jar by stirring (not too vigorously) for 10 secs with a spoon spatula.</p> <ol style="list-style-type: none"> 8. Immediately pour sufficient of the mixture into a centrifuge tube to fill the tube up to the 50 mL mark, that is, until the top level is coincident with the 50 mL mark. 9. Place the centrifuge tube (counterbalanced) in the centrifuge set at 20 °C to 25 °C. 10. Bring the centrifuge as quickly as possible to the rotational frequency, producing an acceleration of 160 g_n at the internal bottom of the tube, and then spin the tube at this rotational frequency for 5 min. 11. Remove the centrifuge tube from the centrifuge and using a spoon spatula, remove and discard any top layer of fatty material in the tube. 12. Hold the centrifuge tube in a vertical position and remove the supernatant liquid with a siphon fitting or a suction tube until the top level is coincident with the 15 mL mark, if a roller-dried product is being tested, or coincident with the 10 mL mark, if a spray-dried product is being tested, taking care not to disturb the sediment. 13. However, if it is evident that the volume of sediment exceeds 15 mL or 10 mL, respectively, discontinue the procedure at this stage and record the insolubility index as “greater than 15 mL” or “greater than 10 mL”, indicating the reconstituting temperature. 14. Otherwise, add water at 24 °C or 50 °C as appropriate to the centrifuge tube until the top level is coincident with the 30 mL mark. 15. Completely disperse the sediment with the stirring rod, tap the bottom of the rod against the inside of the tube to collect adhering liquid and add more water at the same temperature until the top level is coincident with the 50 mL mark. 16. Close the centrifuge tube with a rubber stopper; invert the tube quickly five times so as to mix its contents thoroughly, remove the stopper (draw the bottom of the stopper across the rim of the tube to collect adhering liquid) and spin the tube in the centrifuge for 5 min at the required rotational frequency and temperature. 17. It is recommended that the centrifuge tube be placed in the centrifuge cup so that, when the tube is in the spinning position, the scale lines are not facing upwards or downwards but are midway between these two positions. Then, if the top of the sediment is sloping, the volume of sediment will be easier to estimate. 18. Remove the centrifuge tube from the centrifuge, hold the tube in a
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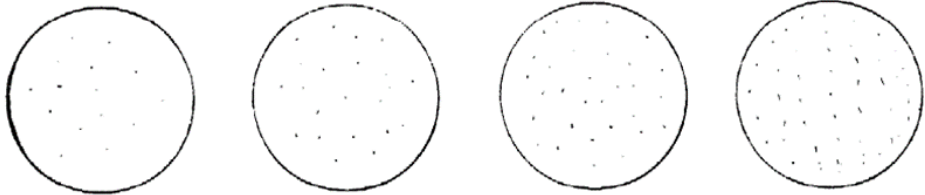
	<p>vertical position against a suitable background with the top of the sediment at eye level and using the magnifying lens, read the volume of sediment to the nearest 0.05 mL if the volume is less than 0.5 mL and to 0.1 mL if the volume is more than 0.5 mL.</p> <p>19. Estimate the volume if the top of the sediment is sloping. If the top of the sediment is uneven, leave the tube in a vertical position for a few min; the top of the sediment will usually become less uneven and the volume of sediment can be read more easily. Record the temperature of the water used for the reconstitution.</p> <p>NOTE: Viewing the tube against either a light or a dark background, as preferred, makes the top of the sediment more distinct.</p>
Calculation with units of expression	<p>The insolubility index of the laboratory sample is equal to the volume, in mL, of sediment recorded. Report the result with the temperature of the water used for reconstitution, for example as follows:</p> <p>a) 0.10 mL (24 °C); and</p> <p>b) 4.1 mL (50 °C).</p>
Reference	<p>IS 12759: 2019. Dried Milk and Dried Milk Products — Determination of Insolubility Index (First Revision). Bureau of Indian Standards, New Delhi</p>
Approved by	<p>Scientific Panel on Methods of Sampling and Analysis</p>

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Solubility Percentage in Foods for Infant Nutrition		
Method No.	FSSAI 01.068:2021	Revision No. & Date	0.0
Scope	This method is for the determination of solubility percentage in infant milk substitutes.		
Caution	Wear laboratory apron, shoes, safety goggles and mask while working in the laboratory.		
Principle	In this method, a definite quantity of powder is reconstituted in water under specified conditions. Reconstituted sample is centrifuged and the fat layer is removed. The sediments are again dissolved and total solids (TS-1) are determined in an aliquot. The sample is again centrifuged and total solids of an aliquot of supernatant layer (TS-2) is determined. Difference in total solids of two (TS-1 and TS-2) provide solubility of powder. The solubility percent is then calculated by taking into account of initial weight of sample taken.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Shallow, flat-bottom dishes of aluminium, nickel, stainless steel, porcelain or silica, 7-8 cm diameter, about 1.5 cm in height and provided with easily removable but closely fitting lids. 2. 50 mL boiling tube. 3. Water bath (50 ± 1 °C). 4. Hot air oven (forced air type), capable of being controlled at 100 ± 2 °C. 5. Centrifuge capable of running at $770 \times g$. 6. Analytical balance (Readability 0.01 g). 7. Aluminium dish with lid. 8. Refrigerator ($4-7$ °C). 		
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation.		
Method of analysis	<p style="text-align: center;">Reconstitution of Milk Powder</p> <ol style="list-style-type: none"> 1. Weigh accurately 4 g of the material into a 50 mL boiling tube, add 32 mL of water at 50 ± 1 °C; cork the tube and shake for 10 sec. 2. Place the tube in a water-bath maintained at 50 ± 1 °C for 5 min. 3. Shake the tube for one min. <p style="text-align: center;">Removal of Fat</p> <ol style="list-style-type: none"> 1. Fill the reconstituted milk into a 25 mL centrifuge tube and centrifuge for 10 min at 2000 rpm with a radius of 17 cm (giving a force of $770 g$). 2. Cool in a refrigerator or in ice until the fat solidifies (taking care that milk does not freeze). 3. Remove the fat layer with a spoon shaped spatula. 		


	<ol style="list-style-type: none"> 4. Bring the milk to room temperature (27 ± 1 °C). 5. Break up the deposit with a glass rod, cork the tube and shake vigorously until the liquid is homogenous. <p>Determination of Total Solids</p> <ol style="list-style-type: none"> 1. Transfer about 2 mL of the homogenous liquid to a previously weighed dry tared aluminium dish (No. 1) (6 cm in diameter and 2.5 cm in height) with a tight-fitting lid. 2. Weigh the dish with the lid on and place it aside. 3. Centrifuge the tube again for 10 min at 2000 rpm with a radius of 17 cm (giving a force of 770 g). 4. Pipette about 2 mL of the upper layer of the supernatant liquid without disturbing the sediment, into a second aluminium dish (No. 2) of the same type as No. 1. 5. Cover the dish and weigh. 6. Uncover both the dishes and place them side by side on a water-bath until dry. 7. Place the dishes in an air-oven at 100 ± 2 °C for 90 min. 8. Cover and transfer the dishes to a desiccator; cool and weigh the dish individually.
<p>Calculations with units of expression</p>	<p>Solubility, percent by weight = $\frac{M_4 \times M_1 \times 100}{M_3 \times M_2}$</p> <p>Where,</p> <p>$M_1$ is Mass in g of the liquid taken immediately after the removal of fat in dish No.1</p> <p>M_2 is Mass in g of the supernatant liquid taken in dish No. 2</p> <p>M_3 is Mass in g of total solids in dish No. 1, and</p> <p>M_4 is Mass in g of total solids in dish No. 2</p>
<p>Reference</p>	<p>IS 14433: 2007 (Reaffirmed year 2018). Infant Milk Substitutes (Annex F, Amendment No. 3). Bureau of Indian Standards, New Delhi.</p>
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>


Determination of Scorched Particles in Dried Milk Products

Method No.	FSSAI 01.068:2021	Revision No. & Date	0.0
Scope	This method is for the determination of scorched particles in whole, skimmed, partially skimmed milk powder, cream powder, milk protein concentrates, dairy permeates, colostrum powder, and dairy whitener.		
Caution	Wear laboratory apron, shoes, safety goggles and mask while working in the laboratory.		
Principle	In this method, dried milk powder after reconstitution with distilled water is passed through a cotton filter disc and dried. The resultant disc is compared with the scorched particle disc test cards.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Scorched particles standard photo prints for dry milks / scorched particles discs test cards. 2. Analytical Balance: Torsion or similar type, approximately 500 g capacity and 0.1 g or better sensitivity. 3. Mixer: Waring blender or similar type. Waring Blender with worn or bent shaft should be replaced immediately since metal particles may be produced, given false scorched particles reading. 4. Scorched particles filtering discs: Cotton discs 3.8 cm (1.25”) diameter or cotton pads mounted on test cards use with the aspirator type tester. 5. Scorched particle tester: Aspirator or pressure type, 3.2 cm (1.125”) filtering diameter. 6. 250 mL measuring cylinder 		
Materials and reagents	<ol style="list-style-type: none"> 1. Defoaming agent: Diglycol laurate S or Antifoam B emulsion. 2. Water: should be sediment free and distilled with temperature between 32.5 and 41 °C. 		
Sample preparation	Refer method FSSAI 01.054:2021 for sample preparation		
Method of analysis	<ol style="list-style-type: none"> 1. Measure 250 mL sediment free water and weigh 25 g of non-fat dry milk or dry butter milk or 32.5 g dry whole milk. 2. Transfer sediment free water and weighed sample to waring blender jar and add approximately 0.5 mL of diglycol laurate S. 3. Mix for 60 secs in the blender and filter the entire solution through a standard cotton disc, using an aspirator or pressure type tester. 4. Rinse the mixing container in tester with approximately 50 mL of sediment free water, also passing this through the cotton disc. 5. If re-liquefied sample is allowed to stand before filtering, stir vigorously just before pouring it into the tester. Do not allow samples to stand uncovered. 6. Remove the filter disc, place it in a scorched particle disc test card and 		

	<p>dry at 30 to 40 °C in a dust free atmosphere.</p> <p>7. Compare the dry disc, placed on a table and viewed from directly above with the scorched particle standard photo prints under uniform, indirect light. Any test falling between two standard discs should be assigned the higher disc's letter.</p> <p>Example: A disc showing more scorched particle than the standard disc A but less than B should be assigned B, and similarly for the other discs.</p> <p>NOTE - for illustrative purpose only, a black and white reproduction of these standard discs is given below. These illustrations shall not be used for official classification of scorched particle content (see Figure)</p> <p style="text-align: center;"> A-7.5 mg B-15 mg C-22.5 mg D-32.5 mg </p>  <p style="text-align: center;">Figure. Scorched particle standards for dry milk (Source IS 13500-1992)</p>
Reference:	IS 13500: 1992 (Reaffirmed 2018). Spray dried milk powder - Scorched particles- determination. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

I. KHOA

	Preparation of Sample of Khoa		
Method No.	FSSAI 01.070:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of khoa.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Grating device. 2. Spatula/Spoon. 3. Airtight container for appropriate size. 		
Sample preparation	<ol style="list-style-type: none"> 1. Take all precautions to ensure proper preservation of the sample and to prevent condensation of moisture on the inside surface of the container. 2. The storage temperature should be below 10 °C. Grind or grate the laboratory sample of khoa by means of an appropriate device; mix the ground or grated mass quickly, and if possible, grind or grate a second time, and again mix thoroughly. 3. If the sample cannot be ground or grated, mix it thoroughly by intensive stirring and kneading. 4. Transfer the test sample to an air-tight container to await analysis, which should be carried as soon as possible after grinding. 		
Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 Determination of Total Solids in Khoa	
Method No.	FSSAI 01.071:2021 Revision No. & Date 0.0
Scope	This method is for the determination of total solids in khoa.
Caution	Follow all safety procedures and wear laboratory apron and safety goggles. 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage.
Principle	The total solids content of khoa is determined by drying the product in hot air oven at 102 ± 2 °C to constant mass.
Apparatus/Instruments	Refer requirement stated for analysis of moisture in cheese (FSSAI 01.038:2021).
Materials and reagents	
Preparation of reagents	
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.
Method of analysis	Refer method prescribed for analysis of moisture in cheese (FSSAI 01.038:2021).
Calculation with units of expression	Total Solids Content (%) = $\frac{M_2 - M}{M_1 - M} \times 100$ Where, M is the mass in g of empty dish along with lid; M ₁ is the initial mass in g of dish with lid and test portion M ₂ is mass in g of dish with lid and dried test portion Round the value obtained to nearest 0.01 % (m/m)
Reference	IS 2785 -1979 (Reaffirmed year 2016). Specification for Natural cheese (Hard Variety), Processed Cheese, Processed Cheese Spread and Soft Cheese. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Fat Content on Dry Matter Basis in Khoa


Method No.	FSSAI 01.072:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat content in khoa.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 5. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 		
Principle	<p>In this method fat is extracted from the sample using either by digesting the protein matrix using hydrochloric acid or ammonia. The process of extraction of fat from khoa depends on whether sugar is present or not. If sugar is present, fat is extracted with Rose Gottlieb, and if not, then fat is extracted by acid digestion method. The liberated fat is extracted using a combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. The addition of ethanol facilitates the passage of the fat from the aqueous phase to the solvents. The total solids in the sample are determined separately. Using calculations, fat content on dry matter basis is then calculated.</p>		
Apparatus/Instruments			

Materials and reagents	Refer requirement stated for analysis of fat content in cheese and dried milk (FSSAI 01.039:2021 and FSSAI 01.056:2021).
Preparation of Reagents	
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.
Method of analysis	<p>Method 1: If sample shows presence of sugar:</p> <ol style="list-style-type: none"> 1. Accurately weigh 2-3 g of the sample in a glass beaker, add 5 mL of warm water and break the lumps with the help of a glass rod. 2. Transfer the contents carefully to a Mojonnier flask or other extraction flask. 3. Wash the beaker and glass rod with warm water, add 1.25 mL of ammonia solution 4. Proceed for the extraction of fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021). <p>Method 2: If sample shows absence of sugar:</p> <ol style="list-style-type: none"> 1. Accurately weigh 2-3 g of sample in a small beaker, add a few drops of water and rub to a smooth paste by using a glass rod. 2. Add 9 mL of water (the first few drops being used to wash the tip of the glass rod). 3. Add 10 mL of concentrated hydrochloric acid and boil gently, with shaking, either over a flame or in boiling waterbath, until all solid particles are dissolved. Cool to room temperature (25±3°C) in running water. Transfer the contents to the Mojonnier fat extraction flask. 4. Proceed for the extraction of fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021) <p>Determination of total solids Refer method of analysis for total solids content in khoa (FSSAI.01.071.2021)</p>
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying M₂ is the mass, in g, of the empty fat-collecting flask M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p>

	<p>w is the weight of the sample in g</p> <p>Total Solids (%) = Refer method of analysis for total solids moisture content in khoa (FSSAI.01.071.2021)</p> $\text{Fat \% (on dry matter basis)} = \frac{\text{Fat (\%)}}{\text{Total Solids (\%)}} \times 100$
Reference	<ol style="list-style-type: none"> 1. IS 4883: 1980 (Reaffirmed year 2019). Specification for Khoa. Bureau of Indian Standards, New Delhi. 2. AOAC 905.02. 21st Edn. (2019). Official method -Fat in milk. AOAC International, USA. 3. Pearson's Composition and analysis of foods, 9th edn, 1991 page 538.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Detection of Starch in Khoa

Method No.	FSSAI 01.073:2021	Revision No. & Date	0.0
Scope	This method is for the determination of starch in khoa.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Iodine: It is not compatible with combustibles, strong bases, halogens and ethanol. It reacts violently or explosively with acetylene, acetaldehyde, metal hydrides and metal carbides. 2. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 		
Principle	In this method, a definite quantity of sample is dispersed in hot water and the diluted sample is treated with iodine solution. The appearance of blue colour confirms presence of starch in khoa.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. 50 mL volumetric flask. 2. 100 mL volumetric flask. 3. Analytical balance (Readability 0.01 g). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Iodine. 2. Potassium iodide. 		
Preparation of Reagents	<ol style="list-style-type: none"> 1. Iodine solution: Dissolve 1 g of iodine and 5 g of potassium iodide in sufficient quantity of water and make up the volume to 100 mL. 		
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take 11.0 g of grated khoa sample in a beaker, add 20 mL of hot water (80 - 90 °C) and with the help of glass rod, make a paste. 2. Transfer the sample quantitatively to 50 mL volumetric flask and make the volume to 50 mL. Shake well before use. 3. Take about 5 mL of prepared sample of khoa in a test tube, add 0.2 mL of iodine solution to the test tube and mix well. 4. Observe the colour. 		
Inference (Qualitative analysis)	Development of blue colour indicates presence of starch and control sample remains yellow. The limit of detection is 0.05%.		
Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		


 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Detection of Sucrose in Khoa		
Method No.	FSSAI 01.074:2021	Revision No. & Date	0.0
Scope	This method is for the detection of sucrose in khoa.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Resorcinol: Avoid using for a prolonged period, on a broken skin. For external use only; avoid contact with eyes, mouth, and nose. Caution should be exercised in patients with any allergy, children, elderly, during pregnancy and breastfeeding. 		
Principle	In this method, a definite quantity of sample is dispersed in hot water and the diluted sample is treated with resorcinol solution. The appearance of red colour confirms presence of sugar in khoa.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. 50 mL volumetric flask. 2. Analytical balance (Readability 0.01 g). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Resorcinol flakes (should be white in colour). 2. Concentrated hydrochloric acid (Approximately 11.6 N). 		
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Preparation of sample: Take 11.0 g of grated khoa sample in a beaker, add 20 mL of hot water (80-90 °C) and with the help of glass rod, make a paste. Transfer the sample to 50 mL volumetric flask and make the volume to 50 mL. Shake well before use. 2. Take about 5 mL of prepared sample of khoa in a test tube, add 0.5 mL of concentrated hydrochloric acid followed by addition of 0.005 g of resorcinol. 3. Mix the contents and place the test tube in boiling water bath for 5 min. 4. Observe the colour. 		
Inference (Qualitative analysis)	Appearance of deep red colour indicates presence of sucrose in sample, whereas control samples will remain light pink.		

Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Titratable Acidity in Khoa

Method No.	FSSAI 01.075:2021	Revision No. & Date	0.0
Scope	This method is for the determination of titratable acidity in khoa.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	A known quantity of sample is neutralized with standardized sodium hydroxide solution with phenolphthalein indicator. The amount of sodium hydroxide required is a function of the amount of natural buffering substances present in the product, and of developed or added acid or alkaline substances.		
Apparatus/Instruments	Analytical balance (Readability 0.01 g).		
Materials and reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Phenolphthalein indicator. 3. Ethanol. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Standard Sodium hydroxide solution – 0.1 N. 2. Phenolphthalein Indicator – Dissolve 1.0 g of phenolphthalein in 100 mL of 95% ethanol. Add 0.1 N Sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 mL. 		
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 2 g of the material in a suitable dish or basin, add 3 mL of hot water and render it to paste; add further 17 mL of hot water washing off any adherents. 2. Add 1 mL of phenolphthalein indicator, shake well and titrate against standard Sodium hydroxide solution; complete the titration in 20 sec. 3. Keep a blank by taking 2 g of material diluted with 20 mL of water in another dish for comparison of colour. 		
Calculation with units of expression	<p>Titratable acidity (as lactic acid) percent by mass = $\frac{9 AN}{w}$</p> <p>Where,</p>		

	A = Volume of standard Sodium hydroxide required for titration N = Normality of Standard Sodium hydroxide solution w = weight of the sample taken for test in g
Reference	IS 4883: 1980 (Reaffirmed year 2019). Specification for Khoa. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Ash in Khoa		
Method No.	FSSAI 01.076:2021	Revision No. & Date	0.0
Scope	This method is for the determination of total ash in khoa.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot crucibles. 2. Hot crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	Sample is kept at 550 ± 20 °C in a muffle furnace and ignited so that all the organic matter will be burnt. The leftover inorganic residue is then weighed and the total ash is determined and expressed as percentage.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Platinum or silica crucible about 70 mm diameter and 25 to 50 mm deep. 2. Muffle furnace: Capable of being controlled at 550 ± 20 °C. 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Safety tongs having long arms. 5. Bunsen burner or electric hot plate. 6. Analytical balance (Readability 0.0001 g). 7. Muffle furnace tongs. 8. Gloves. 		
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 3 g of the khoa sample in the crucible, previously dried in a hot air oven and weighed. 2. Heat the crucible gently on a burner or hot plate at first and then strongly in a muffle furnace at 550 ± 20 °C till grey ash is obtained. 3. Cool the crucible in a desiccator and weigh it; heat the crucible again at 550 ± 20 °C for 30 min. 4. Cool the crucible in a desiccator and weigh. 5. Repeat this process of heating for 30 min, cooling and weighing until the difference between two successive weighing is less than 1 mg. 6. Record the lowest mass. 		
Calculation with units of expression	<p>Total ash % by mass = $\frac{M_2 - M}{M_1 - M} \times 100$</p> <p>Where</p>		


	<p>M is mass in g, of the empty crucible; M₁ is mass in g, of the crucible with the material taken for the test; M₂ is mass in g, of the crucible with ash.</p>
Reference	IS 1165: 2002 (Reaffirmed year 2018). Milk Powder – Specifications. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Estimation of Reichert Meissl Value, Polenske Value and Butyro-Refractometer Reading of Fat Extracted from Khoa

Method No.	FSSAI 01.077:2021	Revision No. & Date	0.0
Scope	This method is applicable for determination of Reichert Meissl (RM) value, Polenske Value and Butyro-Refractometer Reading (BRR) of the fat extracted from khoa.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalies as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	The sample is extracted with light petroleum ether. The solvent is distilled off and the residue dried and weighed.		
Apparatus/Instruments	<ol style="list-style-type: none"> Soxhlet extraction apparatus. Extraction thimbles, free of matter soluble in light petroleum and having porosity consistent with the requirements. Hot air oven (forced air type), capable of being controlled at 100 ± 3 °C. Extraction Cups. Analytical balance (Readability 0.001 g). 		
Materials and Reagents	Light petroleum ether, boiling range: 40 to 60 °C.		
Sample preparation	Refer method FSSAI 01.070:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> Weigh approximately 15 g of the sample (M_1), transfer it to an extraction thimble and cover with a fat-free cotton wool. The sample may be taken in quadruplicate to obtain sufficient quantity of fat (12 g fat may be obtained from 60 g of sample). 		

	<ol style="list-style-type: none"> 2. Place the thimble in extraction cup then place the cup in extractor and pour 150 mL of petroleum ether. 3. Turn on the heating mantle and run the extraction for approximately 4 to 6 h at condensation rate of 5 to 6 drops per second. 4. Collect all petroleum ether in the conical glass flask and evaporate. 5. Place the flasks in hot air oven for approximately two h at 100 °C. 6. Leave the flasks at 25 ± 3 °C to cool. 7. Use the extracted fat for estimation for Reichert Meissl Value & Polenske value (FSSAI 01.089:2021) and Butyo-refractometer reading (FSSAI 01.087:2021) as per the method specified for milk fat products
Calculation with units of expression	Refer calculation as described for Reichert Meissl Value, Polenske value and butyo-refractometer reading in method specified for milk fat products (FSSAI 01.087:2021 and FSSAI 01.089:2021).
Reference	AOAC 948.22 21 st Edn. (2019). Fat (Crude) in Nuts and Nut Products. AOAC International, USA.
Approved by	Scientific Panel on Methods of Sampling and Analysis


J. BUTTER

		Preparation of Sample of Butter	
Method No.	FSSAI 01.078:2021	Revision No. & Date	0.0
Scope	This method specifies the sample preparation of butter for further physical and chemical analysis		
Apparatus/Instruments	<ol style="list-style-type: none">1. Water bath/oven maintained at 37 ± 2 °C.2. Airtight container for appropriate size.		
Sample preparation	<ol style="list-style-type: none">1. Warm the sample in an airtight container with the lid screwed down tightly or with the glass stopper in an oven or water bath maintained at 37 ± 2 °C.2. Shake vigorously to obtain a homogeneous fluid emulsion, free from unsoftened pieces.3. In case the sample does not mix up properly (water separation can be seen), reject the sample.		
Reference	<ol style="list-style-type: none">1. IS 3507 – 1966 (Reaffirmed year2018) Method of sampling and test for Butter. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Determination of Moisture in Butter

Method No.	FSSAI 01.079:2021	Revision No. & Date	0.0
Scope	This method describes the determination of moisture in butter using.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The sample is kept in a hot air oven at 100 ± 1 °C. The moisture content in butter is determined by the reduction in weight of the sample.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Hot air oven (forced air type), capable of being controlled at 100 ± 1 °C. 3. Flat bottom moisture dish: Dishes of height at least 25 mm and at least 50 mm in diameter, and made of appropriate material (for example stainless steel, nickel or aluminium) not affected by boiling water. 4. Glass rods with one end flattened and about 9 cm in length. 5. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 6. Boiling water-bath with rings to take dishes of 50 mm diameter. 7. Clay pipe triangles. 		
Sample preparation	Refer method FSSAI 01.078:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Clean the dish and the glass rod and dry them in the hot air oven maintained at 100 ± 1 °C for at least 1 h. 2. Allow to cool to the room temperature (25 ± 3 °C) in a desiccator and weigh the dish. Accurately weigh (to the nearest 0.1 mg) into the dish 3 to 4 g of the prepared butter sample. 3. Place the dish on a boiling water-bath supported on a clay pipe triangle for at least 20 min, stirring at frequent intervals until no moisture can be seen. Wipe the bottom of the dish and transfer it to the oven maintained at 100 ± 1 °C and keep it for 90 min. Allow the dish to cool in the desiccator and weigh to the nearest 0.1 mg. 4. Heat the dish again in an oven for 30 min. Repeat the process of heating, cooling and weighing until the difference between two consecutive weights does not exceed 0.1 mg. Record the lowest mass and preserve the residue for the determination of curd. 		

	5. Note: As per IDF (IDF 80-1, 2001) procedure, the weight of butter sample taken for moisture determination is 5 g and drying temperature is 102 ± 2 °C for 1 h.
Calculation with units of expression	$\text{Moisture \% by mass} = \frac{M_1 - M_2}{M_1 - M} \times 100$ <p>Where, M is mass in g, of the empty dish along with glass rod; M₁ is initial mass in g of the dish, glass rod and test portion; M₂ is the mass in g of the dish, glass rod and dried test portion Express the results to the nearest 0.01% (m/m).</p>
Reference	<ol style="list-style-type: none"> 1. IS 3507: 1966 (Reaffirmed year 2018). Methods of sampling and test for Butter. Bureau of Indian Standards, New Delhi. 2. AOAC 920.116. 21st Edn. (2019). Official method Moisture in Butter; IDF (2001). AOAC International, USA. 3. IDF Standard 80-1. Butter – Determination of moisture, non-fat solids and fat contents (Part 1): Determination of moisture content (reference method) International Dairy Federation, Brussels.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of Fat and Curd (Milk solids not Fat) in Butter		
Method No.	FSSAI 01.080:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat and curd (milk solids not fat) in butter.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <p>1. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood.</p>		
Principle	In this method, the fat portion is removed using petroleum ether and residue is dried for determination of curd content. In case of table butter, it is curd and salt content, and thus salt content has to be determined separately (Refer method FSSAI 01.082:2021) for calculating curd content.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Gooch crucible or sintered funnel - with filter flask and adapter. 2. Glass funnel with folded 12.5 cm Whatman filter paper Grade 1. 3. Flat bottom flask of 250 mL capacity. 4. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 5. Celite 545 (diatomaceous earth). 6. Hot air oven (forced air type), capable of being controlled at 100 ± 1 °C. 7. Conical flask: 250 mL capacity. 8. Glass beads. 		
Materials and reagents	Petroleum ether – boiling range 40-60 °C		
Sample preparation	Refer method FSSAI 01.078:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Prepare a celite mat in a Gooch crucible or sintered funnel. Dry it in a hot air oven maintained at 100 ± 1 °C, cool in the desiccator and weigh. Alternatively, dry, cool and weigh ordinary glass funnel with folded 12.5 cm filter paper. 2. Melt the residue in the moisture dish (obtained after determination of moisture) and add 25 to 50 mL of petroleum ether and mix well. 3. Fit the crucible to the filter flask or place the funnel with filter paper on a filter stand. 4. Wet the celite mat or the filter paper with petroleum solvent and decant the fatty solution from the dish into the celite or the filter paper, leaving the sediment in the dish. 		

	<ol style="list-style-type: none"> 5. Macerate the sediment twice with 20 to 25 mL of petroleum solvent and decant again the fatty solution into the asbestos or the filter paper. 6. Filter the solution and collect the filtrate in a clean, dried, tared 250 mL flat bottom flask, containing 1 to 2 glass beads. 7. With the aid of a wash-bottle containing petroleum solvent, wash all the fat and sediment from the dish into the crucible or the filter paper. 8. Finally, wash the crucible or the filter paper until free from fat, collecting all the filtrate in the conical flask. Preserve the filtrate for the determination of fat. Dry the crucible or filter paper in the oven maintained at 100 ± 1 °C for at least 30 min. 9. Note: If only fat is to be determined, transfer all the filtrate to a pre-dried and weighed fat flaks containing 2-3 glass beads. Rinse the conical flask with petroleum ether. Evaporate the ether, first on the water-bath and then in the oven at 102 ± 2 °C for 1 h or till the time the constant weight is obtained. Cool in the desiccator and weigh. Repeat drying, cooling and weighing until the loss of weight between the consecutive weighing does not exceed 0.1 mg. Preserve the residue for the determination of salt.
Calculation with units of expression	<p>Calculate the fat content form the residues obtained by using following formula</p> $\% \text{ Fat} = \frac{(\text{Weight of fat flask+fat residues})-\text{Weight of empty flask}}{\text{Weight of sample taken}} \times 100$ <p>Curd and salt</p> $\text{Curd and salt, \% by mass (C)} = \frac{M_1 - M_2}{M} \times 100$ <p>Where, M is mass in g, of the sample M₁ is mass in g, of the filter paper with residue; M₂ is mass in g, of the filter paper alone;</p> <p>Curd in butter</p> <p>Curd percent by weight is obtained by subtracting the value of salt percent by weight from the value of C. Refer method FSSAI 01.082:2021 for determination of salt in butter.</p>
Reference	<ol style="list-style-type: none"> 1. IS 3507: 1966 (Reaffirmed year 2018). Method of sampling and Test for Butter. Bureau of Indian Standards, New Delhi. 2. Pearsons Composition and Analysis of Foods 9thedn, page 576.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Fat in Butter

Method No.	FSSAI 01.081:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fat in butter.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 3. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 5. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	<p>Fat content in butter can be estimated by either Gerber Method or Rose-Gottlieb method. The Gerber method is suitable as a routine or screening test. The butter is mixed with sulphuric acid and iso-amyl alcohol in a special Gerber tube, permitting dissolution of the protein and release of fat. The tube is centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the fat content of butter. This method can be used for routine purposes.</p> <p>Rose-Gottlieb Method is a gravimetric method in which fat globule membrane of milk fat globules is ruptured by addition of ammonia to milk sample to liberate the fat. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. This method is</p>		

	considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.
Apparatus/Instruments	Refer apparatus requirement stated for milk (FSSAI 01.023:2021), except Butyrometer required for analysis of butter would be range up to 70% (ISI marked).
Materials and reagents	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021).
Preparation of Reagents	Refer requirement stated for analysis of fat content in milk (FSSAI 01.023:2021).
Sample preparation	Refer method FSSAI 01.078:2021 for sample preparation.
Method of analysis	<p>Method 1: Gerber Method</p> <ol style="list-style-type: none"> 1. Weigh 2.5 g of sample in a 25 mL beaker and mix it with small portions of 1:1 sulphuric acid. Transfer the contents into the butyrometer with the help of a small funnel. 2. Rinse the beaker about six times with small quantities of dilute sulphuric acid to make sure that all butter has been transferred. 3. Mix 10 mL of water and 10 mL of sulphuric acid in a small beaker; add between 15 to 20 mL of the mixture to the butyrometer depending on its size. 4. Add 1 mL of amyl alcohol, preferably by use of an automatic dispenser. Continue analysis as per method prescribed for analysis of fat in milk by Gerber method (FSSAI 01.023:2021). <p>Method 2: Rose-Gottlieb Method</p> <ol style="list-style-type: none"> 1. Weigh accurately 1 g of butter sample into a 50 mL beaker, add 9 mL of 0.5% sodium chloride solution to disperse and transfer to fat extraction apparatus. 2. Add 1 mL of concentrated ammonia and mix well. 3. Proceed for the extraction fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021).
Calculation with units of expression	<p>Method 1: Gerber Method: See the method of analysis.</p> <p>Method 2: Rose-Gottlieb Method:</p> $Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where, M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying M₂ is the mass, in g, of the empty fat-collecting flask</p>

	<p>M_3 is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M_4 is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Reference	<ol style="list-style-type: none"> 1. IS 3507: 1966 (Reaffirmed year 2018). Method of sampling and Test for Butter. Bureau of Indian Standards, New Delhi. 2. IS 3509: 1966. (Reaffirmed year 2018). Method of sampling and test for cream. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Salt Content in Butter

Method No.	FSSAI 01.082:2021	Revision No. & Date	0.0
Scope	This method is for the determination of salt content in butter.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Nitric acid: It is a corrosive chemical. It is highly reactive and a dangerous explosion hazard. It reacts violently and explosively with most metals and powdered metals (antimony, bismuth etc.), alkaline earth metals (magnesium, calcium etc.), and metal hydrides to form flammable and explosive hydrogen gas. 2. Ferric ammonium sulphate: Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. 3. Nitrobenzene: It is highly flammable; keep away from heat, sparks and open flame. Nitrobenzene is highly toxic (Threshold Limit Value 5 mg/m³) and readily absorbed through the skin. Prolonged exposure, avoid breathing fumes/aerosols. Perform all operations under an effective fume removal device. 4. Silver nitrate: It is corrosive. It is not combustible, but it is a strong oxidizer that enhances the combustion of other substances. 		
Principle	<p>In this method, salt present in the butter sample is extracted with hot water from the dried fat-free residue obtained in moisture determination. The chlorides are precipitated by adding excess of silver nitrate. The unused silver nitrate is titrated with potassium thiocyanate using ferric ammonium sulphate indicator.</p> <p>Reaction</p> $\text{Ag}^+ (\text{excess}) + \text{Cl}^- \longrightarrow \text{AgCl} (\text{solid})$ $\text{Ag}^+ + \text{SCN}^- \longrightarrow \text{AgSCN} (\text{solid})$ $\text{Fe}^{+3} + \text{SCN}^- \longrightarrow [\text{FeSCN}]^{+2} (\text{Reddish brown})$		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Beakers of 100, 250 mL capacity. 2. Volumetric flask of 100 mL, 1 L capacity. 3. Conical flask of 250 mL capacity. 4. Water-bath maintained at 60 to 70 °C. 5. Burette of 50 mL capacity, graduated to 0.1 mL. 		

	<ol style="list-style-type: none"> 6. Pipette capable of delivering 2.0 mL. 7. Measuring cylinder of 100 mL capacity, graduated. 8. Analytical balance (Readability 0.001 g).
Materials and reagents	<ol style="list-style-type: none"> 1. Silver nitrate. 2. Sodium chloride 99.9-100% pure – dried at 140 °C. 3. Nitric acid (Approximately 15.8 N). 4. Ferric ammonium sulphate. 5. Potassium thiocyanate (KCNS). 6. Potassium chromate. 7. Calcium carbonate: Analytical Grade, free from chloride. 8. Nitrobenzene.
Preparation of Reagents	<ol style="list-style-type: none"> 1. Standard silver nitrate solution: 0.05 N, Dissolve slightly more than theoretical quantity (8.7 g per 1 L of water) of silver nitrate (equivalent weight 169.89) in halogen-free water and dilute to volume (1 L) and standardize against standard 0.05 N sodium chloride. 2. Nitric acid: Approximately 5 N. Take 31 mL of concentrated nitric acid and make up the volume to 100 mL with distilled water. 3. Ferric ammonium sulphate indicator solution: Dissolve 50 g of ferric ammonium sulphate $[\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}]$ in 95 mL of water containing 5 mL of 5 N nitric acid. 4. Standard potassium thiocyanate (KCNS) solution (Approx. 0.05 N): Weigh approx. 5.25 g KCNS and dissolve in 1 L water. Allow to stand overnight and filter, if necessary, to get a clear solution, standardize by titration against 0.05 N AgNO_3 and dilute with requisite volume of water to get exactly 0.05 N KCNS solution. 5. Potassium chromate indicator (5%, w/v): Dissolve 50 g of potassium chromate (K_2CrO_4) in 1 L of water. 6. Calcium carbonate: Analytical Grade, free from chloride.
Sample preparation	Refer method FSSAI 01.078:2021 for sample preparation.
Method of analysis	<p>Method 1: (Volhard's Method)</p> <ol style="list-style-type: none"> 1. Extract the salt from the residue of curd and salt by repeated washing of the Gooch crucible or filter paper with hot water, or by placing the crucible or filter paper in a beaker of hot water. 2. Collect the rinsing in a 100 mL volumetric flask, passing the solution through a filter paper. Allow to cool to room temperature (25 ± 3 °C) and make up to volume. 3. Take 25 mL water extract into a 250 mL conical flask, and add an excess (normally 25 to 30 mL) of 0.05 N silver nitrate solution. 4. Acidify with 5N nitric acid; add 2 mL of the indicator solution and 1 mL nitrobenzene. Mix and determine the excess of silver nitrate by


	<p>titration with potassium thiocyanate solution until the appearance of an orange tint, which persists for 15 s.</p> <p>5. In the same manner, determine the equivalent of 25 mL or the added amount of silver nitrate as thiocyanate using the same volumes of reagents and water.</p> <p>Method 2: (Mohr's Method)</p> <ol style="list-style-type: none"> 1. Weigh accurately 5 g of butter sample into the 250 mL conical flask. Carefully add 100 mL of boiling distilled water; mix the contents of the conical flask. Allow to stand with occasional swirling for 5 to 10 min. 2. After cooling to 50 to 55 °C (titration temperature), add 2 mL of potassium chromate solution; mix by swirling. Add about 0.25 g of calcium carbonate and mix by swirling. 3. Titrate at 50 to 55 °C with standard silver nitrate solution while swirling continuously, until the brownish colour persists for half a min. 4. Carry out a blank test with all the reagents in the same quantity except the butter sample. The maximum deviation between duplicate determinations should not exceed 0.02% of sodium chloride.
Calculation with units of expression	<p>Method 1. (Volhard's Method)</p> $\text{NaCl, \% by mass} = \frac{23.38 \times N \times (A - B)}{M}$ <p>Where, N = normality of potassium thiocyanate solution; A = volume in mL, of potassium thiocyanate in blank titration; B = volume in mL, of potassium thiocyanate in the sample titration; and M = mass in g, of the butter sample</p> <p>Method 2. (Mohr's Method)</p> $\text{NaCl, \% by mass} = \frac{5.844 \times N (V_1 - V_2)}{M}$ <p>Where, N = normality of silver nitrate solution (0.1N); V₁ = volume in mL, of silver nitrate used in the sample titration; V₂ = volume in mL, of silver nitrate used in the blank titration; and M = mass in g, of the butter sample.</p>
Reference	IS 3507: 1966 (Reaffirmed year 2018). Methods of sampling and test for butter. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Estimation of Reichert Meissl Value and Butyro-refractometer Reading of Fat Extracted from Butter

Method No.	FSSAI 01.083:2021	Revision No. & Date	0.0
Scope	This method is applicable for determination of Reichert Meissl (RM) value, and Butyro-Refractometer (BR) Reading of the fat extracted from butter.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	In this method, fat from the butter is extracted by heating the sample on hot plate/burner followed by filtration. The extracted fat is further used for determination of RM Value and BR reading.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Flat bottom dish of Stainless steel / nickel / aluminium having 7-8 cm diameter and depth of approximately 2.5 cm. 3. Hot plate / burner. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Light petroleum ether, boiling range: 40 to 60 °C. 2. Funnel. 3. Ordinary filter paper. 		
Sample preparation	Refer method FSSAI 01.078:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take appropriate quantity of butter into a flat bottom dish (20 g butter may yield 15 g fat). 2. Heat the dish on hot plate / burner till the disappearance of foam indicating removal of moisture. 		


	<ol style="list-style-type: none"> 3. Filter the content with ordinary filter paper. 4. Use the extracted fat for estimation for Reichert Meissl Value (FSSAI 01.089:2021), and Butyro-refractometer reading (FSSAI 01.087:2021) as per the method specified for milk fat products.
Calculation with units of expression	The fat extracted in different flask can be used further for determination of Reichert Meissl value and BR value as per the methods specified for milk fat products (FSSAI 01.087:2021 and FSSAI 01.089:2021)
Reference	AOAC 948.22 21 st Edn. (2019). Fat (Crude) in Nuts and Nut Products. AOAC International, USA.
Approved by	Scientific Panel on Methods of Sampling and Analysis

K. MILK FAT PRODUCTS


 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	<h3>Preparation of Sample of Milk Fat Products</h3>		
Method No.	FSSAI 01.084:2021	Revision No. & Date	0.0
Scope	The method describes the sample preparation procedure for milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Water bath. 2. Whatman filter paper- Grade 4. 3. Funnel. 4. Bottles. 5. Moisture determination apparatus. 		
Sample preparation	<ol style="list-style-type: none"> 1. Mix the milk fat product sample in the container in which it is received until homogenous. 2. Carry out this operation in a cool place, away from direct sunlight, and complete it in shortest possible time. 3. In the event of any separation taking place in between, that is, mixing and commencement of the chemical analysis for moisture determination, remix the sample. 4. Use this for determining moisture. 5. After moisture determination, place the container or glass bottle containing milk fat products in a water-bath at a temperature not higher than 50 °C till completely melted. 6. Filter through a dried, fluted open-texture, 15 cm Whatman filter paper Grade 4 with the help of a hot water funnel, directly into receiving bottle. The filtered ghee should be bright and clear. 		
Reference	IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Determination of Moisture in Milk Fat Products

Method No.	FSSAI 01.085:2021	Revision No. & Date	0.0
Scope	This method is applicable for the determination of moisture in milk fat, ghee, butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The moisture content of milk fat product is the loss in mass, expressed as a percentage by mass when the product is heated in a hot air oven at 105 ± 1 °C to constant mass.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Moisture dish: Aluminium, nickel or stainless steel, 7-8 cm in diameter, 2-2.5cm deep provided with tight fitting sip on covers. 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Hot air oven (forced air type), capable of being controlled at 105 ± 1 °C. 		
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 10 g of the sample into a moisture dish which has been dried previously and weighed. 2. Place it in a hot air oven for 1 hour at 105 ± 1 °C. 3. Remove the dish from the oven, cool in a desiccator and weigh. 4. Repeat the process by keeping the dish in the oven for half an hour, cooling and weigh till two successive weighing's do not exceed 1 mg. 		
Calculation with units of expression	$\text{Moisture \% by mass} = \frac{M_1 - M_2}{M_1 - M} \times 100$ <p>Where, M is mass in g, of the empty dish along with glass rod; M₁ is initial mass in g of the dish with lid, glass rod and test portion; M₂ is the mass in g of the dish with lid, glass rod and dried test portion Express the results to the nearest 0.01% (m/m).</p>		
Reference	IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

		Determination of Fat in Milk Fat Products	
Method No.	FSSAI 01.086:2021	Revision No. & Date	0.0
Scope	This method describes the determination of fat in milk fat, ghee, butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	Rose-Gottlieb Method is a gravimetric method in which fat globule membrane of milk fat globules is ruptured by addition of ammonia to cream sample to liberate the fat. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.		
Apparatus/Instruments	Refer requirement stated for determination of fat in milk (FSSAI 01.023:2021)		
Materials and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately 0.5 g of prepared product into a 50 mL beaker, add 9 mL of 0.5% sodium chloride solution to disperse and transfer to fat extraction apparatus. 2. Add 1 mL of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used), mix and shake thoroughly. 		

	3. Proceed for the extraction of the fat by Rose-Gottlieb method as prescribed for milk (Method No FSSAI 01.023:2021) starting with the step indication addition of 10 mL ethyl alcohol onwards.
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Reference	<ol style="list-style-type: none"> 1. IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi. 2. IS 1479 (Part II): 1961 (Reaffirmed Year: 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi. 3. IS 3509: 1966 (Reaffirmed year 2018). Method of sampling and test for cream. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 Determination of Butyro-Refractometer Reading in Milk Fat Products	
Method No.	FSSAI 01.087:2021 Revision No. & Date 0.0
Scope	This method is used to determine the butyro-refractometer (BR) Reading and applicable for milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.
Caution	Wear laboratory apron, shoes, safety goggles and mask while working in laboratory.
Principle	Refractive index is the ratio of the velocity of light in vacuum to the velocity of light in the sample medium; more generally, it is expressed as the ratio between the sine of angle of incidence to the sine of the angle of refraction, when a ray of light of a definite known wavelength (usually 589.3 mμ the mean of the D-line of sodium) passes from air into ghee. The refractive index of ghee may be read on an Abbe refractometer which gives the true refractive index or on a thermostatically controlled butyro-refractometer, which reads on an arbitrary scale (BR reading) at constant temperature.
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Precision butyro-refractometer which is thermostatically controlled and fitted with an accurate thermometer reading from 40 to 50 °C. Check the calibration of the instrument as per the manufacturer's instructions. 2. Device to maintain and control the temperature of prism constant at 40 ± 1 °C. Peltier controlled butyro-refractometer which are commercially available can also be used. 3. Sodium lamp; daylight can also be used if the refractometer has an achromatic compensator. 4. Standard fluid for checking the accuracy of the instrument. Usually, this fluid is provided by the manufacturer for calibration of the refractometer.
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<p>The sample should be rendered optically clear and free from water and suspended impurities, this can be done by using following procedure:</p> <ol style="list-style-type: none"> 1. After moisture determination, place the container or glass bottle containing ghee in a water-bath at a temperature not higher than 50 °C till completely melted. 2. Filter through a dried, fluted open-texture 15 cm Whatman filter paper Grade 4 with the help of a hot water funnel, directly into the receiving bottle. The filtered ghee should be bright and clear. 3. The correctness of the butyro-refractometer shall be tested before carrying out the test with liquid of known refractive index (at 40 °C).

4. Open the double prism of the instrument and place a few drops of the fat sample on prism. The fat sample shall completely fill the space between the two prisms and shall show no air bubbles; close prisms firmly.
5. Allow the instrument to stand for few min before reading is taken so that temperature of ghee sample and instrument is same.
6. The reading shall be taken after ghee has been kept in the prism for 2 to 5 min and after it has been ensured that it has attained constant temperature (40 °C) by taking two or more readings.
7. B.R. reading of ghee decreases with the rise in temperature and vice versa. A correction is needed whenever temperature of the butyro-refractometer is either below or above 40 °C.
8. Method of measurement is based upon observation of position of border line on the scale of the instrument. Hold the sector firmly and move backward or forward until the field of vision is divided into light and dark portion. Line dividing these portions may not be sharp but a band of colours.
9. The colours are eliminated by rotating screw head of compensator until sharp, colourless line is obtained. Read the B.R. of the sample directly on the scale.

Notes- It should be borne in mind that presence of free fatty acids considerably lowers the refractive index.

10. In the homologous series of saturated fatty acids from butyric to stearic, the refractive index rises steeply among the lower members, and flattens out at higher chain lengths. A double bond elevates the refractive index: stearic acid has a lower refractive index than oleic, which in turn has a lower value than linoleic.
11. For conversion of refractive index values into butyro-refractometer reading and vice versa use the following formula (Rangappa and Achaya, 1974) or table (BIS, 1966) as follows:

Inter-conversion of refractive index and butyrometer degrees:

Butyrometer reading in degrees = $42.0 + \text{factor} (\text{observed refractive index} - 1.4538)$

The factor to be used varies with the range of refractive index, as shown below:

Observed refractive index range	Factor
1.4500 - 1.4515	1400
1.4515 - 1.4530	1410
1.4530 - 1.4545	1420

1.4545 - 1.4560	1430
1.4560 - 1.4575	1440

Butyrometer degrees to Refractometer index:

Refractometer index = 1.4538 + factor (Observed B.R. - 42.0)

The factor to be used varies inversely with temperature.

Observed BR reading range	Factor
37.5 - 40.0	0.00072
40.0 - 42.5	0.00071
42.5 - 45.0	0.00070
45.0 - 47.5	0.00069

The refractive index decreases with a rise, and increases with a fall in temperature. If the temperature is not exactly at 40 °C, X is added to the observed reading for each degree above or subtracted for each degree below 40 °C pro rata, where,

X for butyro-refractometer = 0.55

X for Abbe refractometer = 0.000365

Normally, the temperature of observation shall not deviate by more than ± 2 °C

Butyro-refractometer Readings and Indices of Refraction


BR Reading	Refractive Index	BR Reading	Refractive Index	BR Reading	Refractive Index
35.0	1.4488	40.5	1.4527	46.0	1.4565
35.5	1.4491	41.0	1.4531	46.5	1.4569
36.0	1.4495	41.5	1.4534	47.0	1.4572
36.5	1.4499	42.0	1.4538	47.5	1.4576
37.0	1.4502	42.5	1.4541	48.0	1.4579
37.5	1.4506	43.0	1.4545	48.5	1.4583
38.0	1.4509	43.5	1.4548	49.0	1.4586
38.5	1.4513	44.0	1.4552	49.5	1.4590
39.0	1.4517	44.5	1.4555	50.0	1.4593
39.5	1.4520	45.0	1.4558		

	40.0	1.4524	45.5	1.4562		
Calculation with units of expression	The maximum difference between duplicate determinations shall not exceed 0.0002 units for the refractive index and 0.1 for the butyro-refractometer reading.					
Reference	<ol style="list-style-type: none"> 1. IS 3507: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi. 2. Rangappa, K.S. & Achaya, K.T. (1974). Indian Dairy Products. Asia Pub. House, Bombay. 					
Approved by	Scientific Panel on Methods of Sampling and Analysis					

Determination of Free Fatty Acids in Milk Fat Products

Method No.	FSSAI 01.088:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of free fatty acids in milk fat, ghee, butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalies as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 3. Potassium hydroxide: It is highly corrosive. It reacts violently with strong acids. While making solutions add pellets to water and not vice versa. 		
Principle	<p>The FFA present in milk fat products is estimated by acid-base titration with alkali (Sodium hydroxide) using phenolphthalein as an indicator and the end point comes at around pH 8.3 (BIS, 1966) and expressed as % oleic acid</p> $\text{RCOOH} + \text{NaOH} \longrightarrow \text{RCOONa} + \text{H}_2\text{O}$		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Conical flasks of 250 mL capacity. 3. Burette: 50 mL, graduated to 0.1 mL. 4. Pipettes. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Ethanol or rectified spirit: 95% (v/v), sp. gr. 0.816, neutral to phenolphthalein. 2. Sodium hydroxide or potassium hydroxide. 3. Potassium hydrogen phthalate. 4. Phenolphthalein indicator. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide or potassium hydroxide: 0.1 N aqueous solution accurately standardized potassium hydrogen phthalate. 2. Phenolphthalein indicator: 1.0% solution in 95 % (v/v) ethanol or rectified spirit. 		
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh 10 g of the milk fat sample in a 250 mL conical flask. 		

	<ol style="list-style-type: none"> 2. In another flask, bring 50 mL of ethanol to the boiling point and while still above 70 °C, neutralize it with 0.1 N sodium hydroxide using phenolphthalein (using 0.5 mL). 3. Add the neutralized alcohol to flask containing milk fat sample and mix the contents of the flask. 4. Bring the mixture to boil, and while it is still hot, titrate with standardized 0.1 N sodium hydroxide, shaking vigorously during the titration. 5. The end point of the titration is reached when the addition of a single drop produces a slight, but a definite colour change persisting for at least 15 s.
Calculation with units of expression	<p>Free fatty acids: is expressed as the percentage of free fatty acids in the sample, calculated as oleic acid, using the following formula:</p> $\text{Free fatty acids (as \% oleic acid)} = \frac{T}{w} \times 2.82$ <p>Where, T is volume of 0.1 N alkali required for titration in mL; w is mass in g, of milk fat sample taken.</p>
Reference	1. IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of Reichert-Meissl and Polenske Value in Milk Fat Products		
Method No.	FSSAI 01.089:2021	Revision No. & Date	0.0
Scope	This method is used to determine the Reichert-Meissl and Polenske value in milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalies as solids or concentrated solutions. While making solution add pellets to water and not <i>vice versa</i>. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 		
Principle	<p>The fat is saponified using glycerol-potash diluted with water and acidified, and thereafter steam distilled in a glass apparatus (Polenske distillation apparatus) at a controlled rate. The condensed and cooled distillate is filtered; the water-soluble acids which pass through are estimated by titration with alkali to give the R.M. value, while the water-insoluble acids collected on the filter paper are dissolved out in alcohol and titrated to give the Polenske value.</p> <p>Reichert-Meissl value is the number of mL of 0.1 N aqueous alkali solution required to neutralize the water-soluble, steam volatile fatty acids distilled from 5 g of milk fat under the precise conditions specified in the method.</p> <p>The PV is the number of mL of 0.1 N aqueous alkali solution required to neutralize the water-insoluble, steam volatile fatty acids distilled from 5 g of milk fat under the precise conditions specified in the method.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Graduated cylinders: 25 mL and 100 mL capacities. 2. Pipette: 50 mL capacity. 3. The assembly of the apparatus for the distillation: 4. Flat-bottom boiling flask (Polenske): The flask shall be made of heat- 		

resistance glass and shall conform to the following details:

- a. Volume contained to bottom of neck: 310 ± 10 mL
- b. Length of neck: 75 ± 5 mm
- c. Internal diameter of neck: 21 ± 1 mm
- d. Overall height: 160 ± 5 mm
- e. Diameter of base: 45 ± 5 mm
- f. Still-head: The still-head shall be made of glass tubing of wall thickness 1.25 ± 0.25 mm, and shall conform to the shape shown in Figure 5.3, and with the following dimensions:

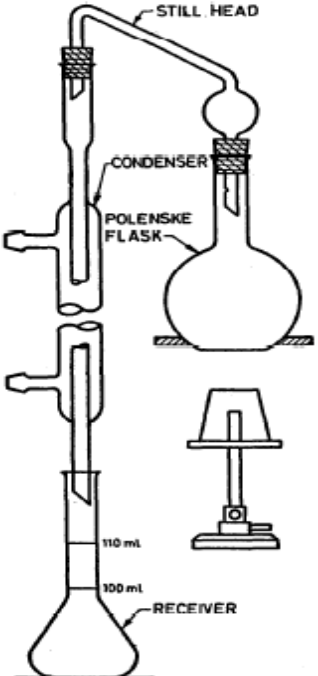
A	180 ± 5 mm
B	107.5 ± 2.5 mm
C	80 ± 5 mm
D	70 ± 5 mm
E	20 ± 2 mm
F	4 ± 1 mm
G (external diameter of bulb)	37.5 ± 2.5 mm
Internal diameter of tubing	8.0 ± 0.5 mm
Acute angle between sloping part of still-head and vertical	$60 \pm 2^\circ$

A rubber stopper, fitted below the bulb of the longer arm of the still-head, and used for connecting it to the flask shall have its lower surface 10 mm above the centre of the side hole of the still-head.

- a. Condenser: The condenser shall be made of glass and conform to the following dimensions:

Overall length	520 ± 5 mm
Length of water jacket	300 ± 5 mm
Length of widened part above water jacket	70 ± 10 mm
Wall thickness of widened part	1.25 ± 0.25 mm
Internal diameter of widened part	20 ± 1 mm
External diameter of inner tube within water jacket	12 ± 0.5 mm
Wall thickness of inner tube	1.0 ± 0.2 mm
Wall thickness of outer jacket	1.25 ± 0.25 mm
External diameter of water jacket	30 ± 2 mm


- b. Receiver: The receiver shall be a flask with two graduation marks on the neck, one at 100 mL and the other at 110 mL.

	<p>c. Asbestos-board: An asbestos-board of 120 mm diameter and 6 mm in thickness, with a circular hole of about 65 mm in diameter shall be used to support the flask over the burner. During distillation, the Polenske flask shall fit snugly into the hole in the board to prevent the flame from impinging on the surface of the flask above the hole. A new asbestos-board may conveniently be prepared by bevelling the edge of the hole, soaking in water, moulding the edge with a flame, and drying.</p> <p>d. Gas burner: The burner should be sufficiently large to allow the distillation to be completed in the time specified in the procedure.</p> <p>The apparatus shall be supported on a retort stand.</p> <p>A. Glass Funnel: Of approximate diameter 6 cm.</p>	 <p>Figure. Apparatus for determination of R.M. Value of Ghee (IS 3508:1966)</p>
<p>Materials and Reagents</p>	<ol style="list-style-type: none"> 1. Glycerol 98% (m/m). 2. Sodium hydroxide. 3. Concentrated sulphuric acid (specific gravity 1.84). 4. Ethanol 95% (v/v). 5. Glass beads, approximately 1.5 to 2.0 mm in diameter. 6. Phenolphthalein indicator. 7. Sodium hydroxide. 	
<p>Preparation of reagents</p>	<ol style="list-style-type: none"> 1. Sodium hydroxide solution (50% (m/m)): Dissolve sodium hydroxide in an equal weight of water and store the solution in a bottle protected from carbon dioxide. Use the clear portion free of the solution from deposit. 2. Dilute sulphuric acid: Use 1 N sulphuric acid (H₂SO₄) prepared by adding 27 mL of concentrated sulphuric acid in 1 L distilled water. Adjust so that 40 mL of 1 N sulphuric acid neutralize 2 mL of the 50% sodium hydroxide solution. 3. Ethanol: 95% (v/v), neutralized to phenolphthalein immediately before use, or neutralized denatured spirit. 4. Phenolphthalein indicator: 0.5% solution in 95% (v/v) ethanol or rectified spirit. 5. Sodium hydroxide solution: Approximately 0.1 N aqueous solution of 	


	Sodium hydroxide of accurately determined strength.
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh 5.00 ± 0.01 g of fat sample into a Polenske flask, add 20 g of glycerol and 2 mL of 50% Sodium hydroxide solution. 2. Heat the flask over a direct flame using a Bunsen burner, with continuous mixing, until the fat, including any drops adhering to the upper parts of the flask is saponified and the liquid becomes perfectly clear; avoid overheating during this saponification. Cover the flask with a watch-glass. 3. Make a blank test without ghee, but using the same quantities of reagents and following the same procedure, again avoiding overheating; such overheating would be indicated by darkening of the solution. Measure 93 mL of boiling distilled water, which has been vigorously boiled for 15 min, into a 100 mL graduated cylinder. 4. When the soap is sufficiently cool to permit addition of the water without loss, but before the soap has solidified, add water, draining the cylinder for 5 secs, and dissolve the soap. If the solution is not clear (indicating incomplete saponification), or is darker than yellow (indicating overheating), repeat the saponification with a fresh sample of ghee. 5. Add two glass beads, followed by 50 mL of the dilute sulphuric acid, and connect the flask at once with the distillation apparatus. 6. Heat the flask without boiling its contents, until the insoluble acids are completely melted, then increase the flame and distil 110 mL in between 19 and 21 min. Keep the water flowing in the condenser at a sufficient speed to maintain the temperature of the issuing distillate between 18 and 21 °C. 7. When the distillate reaches the 110 mL mark, remove the flame and replace the 110 mL flask by a cylinder of about 25 mL capacity, to collect drainings. Close 110 mL flask with its stopper and without mixing the contents; place it in water at 15 °C for 10 min so as to immerse the 110 mL mark. Remove the flask from the water, dry from outside, and invert the flask carefully, avoiding wetting the stopper with insoluble acids. Mix the distillate by four or five double inversions, without violent shaking. 8. Filter through a dry 9 cm open-texture filter paper (Whatman filter paper Grade 4) which fits snugly into the funnel. Reject the first runnings and collect 100 mL in a dry volumetric flask; cork the flask and retain the filtrate for titration. 9. Detach the still-head and wash the condenser with three successive 15


	<p>mL portions of cold distilled water, passing each washing separately through the cylinder, the 110 mL flask, the filter and the funnel, nearly filling the paper each time and draining each washing before filtering the next. Discard the washings.</p> <p>10. Dissolve the insoluble acids by three similar washings of the condenser, the cylinder, and the filter, with 15 mL of neutralized ethanol, collecting the solution in the 110 mL flask and draining the ethanol after each washing. Cork the flask, and retain the solution for titration (for Polenske value).</p> <p>11. Reichert-Meissl or soluble volatile acid value: Pour 100 mL of the filtrate (obtained in step F) containing the soluble volatile fatty acids into a titration flask, add 0.1 mL of phenolphthalein indicator and titrate with 0.1 N sodium hydroxide solution until the liquid becomes pink.</p> <p>12. Polenske or insoluble volatile acid value: Titrate the alcoholic solution of the insoluble volatile acids after addition of 0.25 mL of phenolphthalein indicator, with the 0.1 N sodium hydroxide solution until the solution becomes pink.</p>
<p>Calculation with units of expression</p>	<p>Reichert-Meissl value = $11 \times (\text{Actual Normality of sodium hydroxide}) \times (T_1 - T_2)$</p> <p>Polenske value = $10 \times (\text{Actual Normality of sodium hydroxide}) \times (T_1 - T_2)$</p> <p>Where,</p> <p>$T_1$ = volume in mL of 0.1 N sodium hydroxide solution used for sample under step I;</p> <p>T_2 = volume in mL of 0.1 N sodium hydroxide solution used for blank under step I;</p> <p>T_3 = volume in mL of 0.1 N sodium hydroxide solution used for sample under step J,</p> <p>T_4 = volume in mL of 0.1 N sodium hydroxide solution used for blank under step J;</p> <p>Polenske values, and to a much slighter extent Reichert-Meissl values, have been found to be low when determined at low barometric pressures, such as may occur at high altitudes. The following factors may be applied to values determined at a barometric pressure to convert them to the values determined at normal pressure.</p> <p>Correct Reichert Value = $\frac{(\text{Observed value} - 10) \log 760}{\log P} + 10$</p> <p>Corrected Polenske Value = $\text{Observed value} \times \frac{760 - 45}{P - 45}$</p>

	<p>Where,</p> <p>P =barometric pressure in mm of mercury at the place and time of determination.</p> <p>Reichert-Meissl Value: The maximum deviation between duplicate determinations shall not exceed 0.5 units.</p> <p>Polenske Value: The maximum deviations between duplicate determinations shall not exceed 0.3 units.</p>
Reference	IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Test for Detection of Vanaspati in Milk Fat Products (Baudouin Test)		
Method No.	FSSAI 01.090:2021	Revision No. & Date	0.0
Scope	This method is applicable for the detection of vanaspati in milk fat, ghee, butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 2. Furfural: Keep away from heat, sparks and open flame. It reacts violently with strong oxidizers. Perform all operations under an effective fume removal device. 		
Principle	Addition of sesame (til) oil to vanaspati is mandated by FSSAI. Therefore, detection of sesame oil in milk fat is used as a marker to detect the adulteration of milk fat with vanaspati. Sesame oil contains sesamol and sesamol, which are used to indirectly determine the presence of vanaspati. Sesamol on condensation with furfural produces a pink colour which is used to detect vanaspati.		
Apparatus/Instruments	1. 25 mL measuring cylinder.		
Materials and Reagents	<ol style="list-style-type: none"> 1. Concentrated hydrochloric acid (Approximately 11.6 N). 2. Furfural. 3. Ethanol. 		
Preparation of reagents	1. Furfural solution: 2% solution of furfural, in ethanol, distilled not earlier than 24 h prior to the test from rectified spirit.		
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take 5 mL of the melted ghee in 25 mL measuring cylinder provided with a glass stopper, add 5 mL of hydrochloric acid and 0.4 mL of furfural solution. Insert the glass stopper and shake vigorously for 2 min. 2. Allow the mixture to separate. 3. Observe the colour in the acid layer. 		

Inference (Qualitative analysis)	The development of a pink or red colour in the acid layer indicates presence of vanaspati. Confirm by adding 5 mL of water and shaking again. If the colour in acid layer persists, vanaspati is present. If the colour disappears, it is absent.
Reference	IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Test for Detection of Mineral Oil in Milk Fat Products (Holde's test)		
Method No.	FSSAI 01.091:2021	Revision No. & Date	0.0
Scope	This method is used to detect the presence of mineral oil in milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Potassium hydroxide is a highly corrosive chemical and contact can irritate and burn the skin and eyes 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	Unlike oils and fats, mineral oils are not saponifiable by alkali. This characteristic behaviour of mineral oils is used as the basis for their detection in ghee. Known as Holde's test, the presence of as little as 0.3 % of mineral oil in ghee is detected by saponifying the fat sample and mixing the hot soap solution with water. The appearance of turbidity indicates the presence of mineral oil in the sample (Winton and Winton, 1999).		
Apparatus/Instruments	Flat-bottom flask, 250 mL capacity, fitted with reflux condenser.		
Materials and Reagents	<ol style="list-style-type: none"> 1. Potassium hydroxide. 2. Ethanol. 		
Preparation of reagents	Alcoholic potassium hydroxide (4%): Dissolve 4 g of potassium hydroxide in approximately 10 mL water and make the final volume to 100 mL with ethanol (95%, v/v). The solution should be colourless or very pale yellow. Store this solution in a dark place.		
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Saponify 1 g of the fat with 25 mL of 4% alcoholic potassium hydroxide for about 1 h. 2. Cool and add 100 mL hot (just boiled) distilled water. 3. Appearance of turbidity indicates the presence of mineral oil. 		
Inference (Qualitative analysis)	Appearance of turbidity indicates the presence of mineral oil.		
Reference	<ol style="list-style-type: none"> 1. Kumar, A.; Lal, D.; Seth.; Sharma. V. (2005) Turbidity test for detection of liquid paraffin in ghee. Indian Journal of Dairy Science. 58(4): 298 2. Winton, A.L. and Winton, K.B. (1999) Techniques for Food Analysis. Allied Scientific Publishers, Bikaner, p. 358. 		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Test for Determination of Fatty Acid Composition in Milk Fat Products by Gas-Liquid-Chromatography (GLC)		
Method No.	FSSAI 01.092:2021	Revision No. & Date	0.0
Scope	This method is for the determination of fatty acid composition in milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil. by gas-liquid-chromatography (GLC).		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Methanol: It is a highly flammable liquid and vapour. Keep away from heat, sparks, open flames, hot surfaces. Perform all operations under a fume hood. 2. Potassium hydroxide: Avoid formation of dust; avoid breathing dust, vapours, mist, gas. Use local exhaust ventilation to control airborne levels below recommended exposure limit. 3. n-hexane: Avoid storage near extreme heat, ignition sources or open flame. Use respiratory protective device against the effect of fumes/aerosols. Perform all operations under an effective fume removal device. 		
Principle	<p>The fatty acid composition of milk fat is defined as mass fraction of individual fatty acids as free acid expressed as g/100 g of total fatty acids. Gas liquid chromatography (GLC) has become a useful tool in the quantitative analysis of the fatty acid composition of natural lipids. This method (IDF, 1999a, b) involves base-catalyzed methanolysis of the glycerides in an essentially non- alcoholic solution. After a certain reaction time, the mixture is neutralized by addition of crystalline NaHSO₄ to avoid saponification of pre-formed esters. Fatty acid methyl esters (FAME) are separated and determined by capillary gas-liquid-chromatography (GLC). Quantification of individual FAME in the test sample is done by reference to a milk fat with known composition.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Test tube: 16 mm x 100 mm, fitted with PTFE-lined screw cap. 2. Graduated pipettes of 1 mL and 10 mL capacity. 3. Vortex mixer. 4. Centrifuge, as used for the butyrometric determination of milk fat. 5. Gas-liquid chromatography: The instructions given relate to the usual equipment for gas-liquid chromatography, employing a suitable injection system, a capillary column and a flame ionization detector (FID). 		

6. Injector: Injectors of the vaporizing type (split injector or programmed temperature injector, PTV) that are maintained at least at 220 °C (in the case of a PTV, final injector temperature should be at least 220 °C) or, in the case of a cold on-column injector, the temperature should be maintained some degrees below the boiling point of the solvent.
 7. Oven, capable of being temperature-programmed from near ambient temperature up to 260 °C.
 8. Columns: Columns made of deactivated glass or fused silica with an inner diameter of 0.20 mm to 0.53 mm, a length of 10 m to 50 m and a stationary phase film thickness of 0.2 µm to 1.0 µm. The stationary phase is of the nitroterephthalic acid-modified or unmodified polyethyleneglycol type (for example Carbowax 20M, Supelcowax 10, DB-Wax, Stabilwax, HP-20M, Superox II, CP-Wax 52CB, BP-20, FFAP, SP-1000, DB-FFAP, CP-Wax 58CB).
 9. Flame ionization detector capable of being heated to a temperature 20 °C above the final temperature of the column oven.
 10. Carrier gas pneumatics of the oxygen-diffusion proof type. The column head pressure should be maintained to give a linear carrier gas velocity of 15 to 25 cm/s for nitrogen, 25 to 35 cm/s for helium, and 35 to 55 cm/s for hydrogen at the initial oven temperature. If a vaporizing split injector is used, the split vent flow is controlled to give a split-ratio of 1:50 to 1:100.
 11. Syringe of the 'plunger-in-barrel' type, with maximum capacity of 10 µl, graduated to 0.1 µl.
 12. Computing integrator, capable of baseline indication and reintegration.
- Note:**
13. Performance specification of the GLC: Prepare a test mixture consisting of methyl butyrate, methyl stearate and methyl oleate at a concentration of 0.1 mg/mL each in the fat solvent. Separate this mixture by gas chromatography using the same operating conditions as applied to milk fat samples. The maximum of a test substance peak should rise to approximately three-quarters full scale of the computing integrator. Largest possible separation of methyl butyrate from the solvent peak and a resolution R of 1.5 (baseline resolution) between methyl stearate and methyl oleate should be obtained. R is calculated as:
 14. $R = 2d / (W_1 + W_2)$
- Where,
- a. W_1 and W_2 are the widths, in mm, of the peaks for methyl stearate and methyl oleate, measured between the points of

	<p>intersection of the tangents at the inflexion points of the curve with the baseline;</p> <p>b. d is the distance, in mm, between the respective peak maxima for methyl stearate and oleate.</p> <p>15. Operating conditions for GLC: Select operating conditions, that is, column length and inner diameter, stationary phase film thickness, initial oven temperature, temperature programme rate(s), final oven temperature, carrier gas flow rate to fulfill the specifications as described above in performance specifications of GLC.</p>
<p>Materials and Reagents</p>	<p>Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.</p> <ol style="list-style-type: none"> 1. n-alkane (n-pentane, n-hexane or n-heptane). 2. Methanol, containing not more than 0.5% (m/m) water. 3. Sodium hydrogen sulfate monohydrate (NaHSO₄.H₂O). 4. Potassium hydroxide (approximately 2 N methanolic solution) 5. Reference fatty acid methyl esters: For identification purposes. The reference FAME set should be of high purity (> 90%) and should consist, at least, of the saturated, even numbered, straight chain fame having 4 to 22 carbon atoms in addition to oleic, linoleic and linolenic acid methyl ester. 6. Reference milk fat: For quantification purposes use a milk fat with known fatty acid composition (for example reference material CRM 164, Community Bureau of Reference, Commission of the European communities, Brussels, Belgium). 7. Fat solvent: An appropriate n-alkane (pentane, hexane, heptane) or dichloromethane. 8. Carrier gas: Hydrogen, helium or nitrogen, purity at least 99.999%. The oxygen content should be < 2 ppm. 9. Other gases: Nitrogen and hydrogen, purity at least 99,995%, free from organic impurities (C_nH_m < 1 ppm); synthetic air, free from organic impurities (C_nH_m < 1 ppm). 10. Transesterification reagent, potassium hydroxide or sodium methoxide, methanolic solution of approximately 2 mol/l. Dissolve 11.2 g of potassium hydroxide in 100 mL of methanol and mix well. Alternatively, dissolve 10.8 g of sodium methoxide in 100 mL of methanol and mix well. The sodium methoxide methanolic solution may also be prepared by dissolving 4.6 g of metallic sodium in methanol or by diluting a commercially available solution of approximately 5.4 mol/l.

Preparation of reagents	11. Potassium hydroxide (approximately 2 N methanolic solution): Dissolve 11.2 g potassium hydroxide in 100 mL methanol. Alternatively, sodium methoxide, approximately 2 N methanolic solution may be used. Prepare this solution by dissolving 10.8 g methoxide in 100 mL methanol.
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<p>Step A: Preparation of fatty acid methyl esters of milk fat</p> <ol style="list-style-type: none"> 1. Weigh to the nearest 5 mg, 100 mg of the prepared milk-fat sample in a test tube. 2. Dissolve the sample in 5 mL solvent (n-alkane) and mix, add 0.2 mL transesterification reagent, cap the tube and mix the contents vigorously by means of the vortex mixer for 1 min. 3. After an additional reaction time of 5 min, add 0.5 g solid sodium sulphate and mix. Centrifuge the sample for 3 min at room temperature (25±3°C). An aliquot from the clear supernatant is taken for gas chromatographic analysis. 4. The ester solution may be decanted and stored at refrigeration temperatures or, preferably, in a deep-freezer for several days. Precautions have to be taken to avoid losses due to the volatility of milk fat fatty acid methyl esters. <p>Notes: Sodium methoxide in methanol may be prepared by dissolving 4.6 g metallic sodium in methanol. Special precautions must be taken when handling metallic sodium.</p> <p>n-acyl lipids (for example sphingolipids) are not properly transesterified by base-catalyzed methanolysis. These substances occur primarily in lipids of whey derived products.</p> <p>Alternative procedure for the preparation of FAME using acid catalysed transesterification of glycerides: This method is suitable for the analysis of partially lipolysed milk fat (fat acidity greater than 1 mmol free fatty acids per 100 g of milk fat). In such a case, the following method for the preparation of methyl esters of milk fat should be followed. In this method, the partially hydrolyzed milk fat is converted to FAME by acid-catalysed transesterification of glycerides bound acids with concomitant esterification of free fatty acids:</p> <ol style="list-style-type: none"> 1. Weigh to the nearest 0.10 g, 2.0 g of the prepared ghee sample in an ampule, add 0.4 mL transesterification reagent. Flame seal the ampule. 2. Heat the ampule for 3 h in an oven maintained at 105 °C with intermittent mixing of the contents. Cool to room temperature (25 ± 3 °C) and allow for complete phase separation.

3. An aliquot of the upper phase is taken for further analysis. Dilution of the esters formed with an appropriate solvent may be necessary.

Notes: Substitution of the ampule with another suitable derivitization vial or similar dimension is possible, provided leak-tight closure prevents losses of volatile esters.

Instead of a hot air oven, a boiling water-bath may be used.

Step B: Determination of fatty acid composition by GLC

1. **Sample injection:** In general, follow the manufacturer's instructions for the injector employed. In the case of a vaporizing split injector, the following guidelines should be obeyed-

2. A 0.5 μ l to 1.5 μ l test portion fatty acid methyl ester (FAME) is drawn up with a micro syringe.

3. Withdraw the sample into the barrel of the syringe.

4. Insert the needle into the heated injector and after a dwell time of 3 secs to 5 sec, the plunger is depressed rapidly.

5. Immediately thereafter, the syringe needle is removed from the injector. If sampling is done by the cold on-column technique, the test portion is diluted sufficiently with solvent (n-alkane; for example, 1:10) and injected at an oven temperature at or some degrees below the boiling point of the solvent used.

a. **Qualitative analysis:**

1. Analyze reference FAME under the same working conditions as used for the test sample. Note the retention times of the reference substances. Esters elute in order of increasing number of carbon-atoms and in order of increasing number of double bonds for a given number of carbon-atoms, for example methyl palmitate elutes before methyl stearate; fame with 18 carbon-atoms elute in the order: methyl stearate, methyl oleate, methyl linoleate, methyl linolenate. Branched chain FAME elute before the straight chain ester with the same number of c-atoms in the order: iso-branched before anteiso-branched. The chromatogram given in Figure 5.10 is a further aid for the tentative identification of milk fat FAME. Identify the peaks of the test sample by comparison with the retention data obtained with the reference mixture and by comparison with the chromatogram in the Figure below.

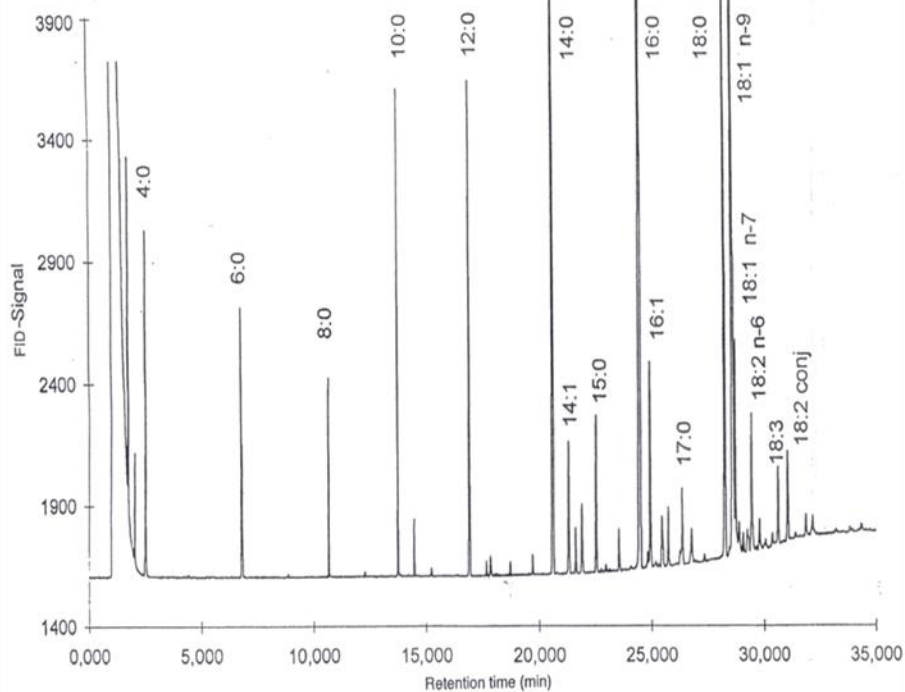


Figure: Separation of milk fat fatty acid methyl ester by GLC
(Source: IDF 184: 2002)

b. Quantitative analysis

1. Determination of correction factors: Transmethylate the reference milk fat as described above (See Step A for the preparation of FAME), and analyze the obtained fame mixture under the same working conditions as used for the test sample. Terminate recording the chromatogram after complete elution of behenic acid (C22:0). To estimate the percentage of a component represented by a peak in the chromatogram, use the method of normalization, which assumes that all components of the sample are represented in the chromatogram, so that the sum of the area under the relevant peaks represents 100% of the sample constituents (100% elution). Determine the areas of peaks attributable to fame (all peaks in the chromatogram except the solvent peak, peaks due to addition of stabilizing agents and those which appear also in blank runs) by using the computing integrator. Calculate the percentage of the area represented by the peak of component i by:

Percent area of component A_i ($A_i\%$) =

$$\frac{A_i}{\sum A_i} \times 100$$

Where A_i is the area of the peak corresponding to component i, and $\sum A_i$ is the sum of the areas under all peaks.

To determine correction factors CF_i which are used to convert the percentages of peak areas into mass percentages of components, the known mass percentage ($m_i\%$) of the component i in the reference milk fat is divided by the area percentage of the corresponding peak in the calibration run:

$$CF_i = \frac{(m_i\%)}{(A_i\%)} = \frac{m_i \times \sum A_i}{\sum m_i \times A_i}$$

Where m_i is the mass (expressed as free fatty acid) of component i and $\sum m_i$ is the total of the masses (expressed as free fatty acids) of the various components in the reference milk fat.

Individual CF_i values are standardized with respect to the CF value of methyl palmitate (relative $CF'_{\text{palmitate}} = 1$) to obtain the relative correction factor of component i (CF'_i):

$$CF'_i = \frac{CF_i}{CF_{\text{palmitate}}}$$

ii. Test sample: Analyze the transmethylated test sample and determine peak areas as described in quantitative analysis of reference fat. Mass percentages of individual components expressed as g free fatty acid per 100 g of total fatty acids are obtained by:

$$\text{Percent mass of component } i = \frac{CF'_i \times A_i \times 100}{\sum (CF'_i \times A_i)}$$

Unidentified sample components (“unknown”) are included in the final report and a CF' value of 1 is used for calculations.

Notes:

1. Instead of injecting samples manually, an automatic sample may be used.


The stationary phase specified in apparatus (specification of column) may be substituted by other polar phases provide that a similar resolution of FAME is obtained.

Method 2

(AOAC 996.06 21st Edn. 2019)

Fat and fatty acids are extracted from food by hydrolytic methods (acidic hydrolysis for most products, alkaline hydrolysis for dairy products, and combination for cheese). Fat is extracted into ether, then methylated to fatty acid methyl esters (FAMES) using BF_3 in methanol. FAMES are quantitatively measured by capillary gas chromatography (GC) with Flame Ionization Detector (FID). Results of different individual or category of

	fatty acids are calculated based upon peak area of individual fatty acids and combining them respectively. FAME mixture of 37 fatty acids is used for identification.
Calculation with units of expression	As mentioned in the Method of Analysis
Reference	<ol style="list-style-type: none"> 1. IDF (2002) IDF Standard 182, Milk fat. Preparation of fatty acid methyl esters. International Dairy Federation, Brussels; 2. IDF (2002) IDF Standard 184, Milkfat. Determination of the fatty acid composition by gas-liquid chromatography. International Dairy Federation, Brussels.) 3. AOAC 996.06 21st End. 2019 Fatty Acids in Oils and Fats
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Peroxide Value in Milk Fat Products		
Method No.	FSSAI 01.093:2021	Revision No. & Date	0.0
Scope	This method is applicable for determining the of peroxide value of milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Chloroform: Inhaling chloroform vapors may cause drowsiness or dizziness. Use in a well ventilated hood Perform all operations under a fume hood. 2. Potassium iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 		
Principle	<p>The usual method of assessment of rancidity in ghee is by determination of peroxide value (PV) which is reported in units of milliequivalents of peroxide oxygen per kg of fat or mL of 0.002 N sodium thiosulphate per g of sample. The most common method for PV determination is based on iodometric titration which measures the iodine produced from potassium iodide by the peroxides present in a fat or oil.</p> $\text{ROOH} + \text{KI (in excess)} \longrightarrow \text{ROH} + \text{KOH} + \text{I}_2$ $\text{I}_2 + \text{starch} + 2\text{Na}_2\text{S}_2\text{O}_3 \longrightarrow 2\text{NaI} + \text{starch} + \text{Na}_2\text{S}_4\text{O}_6$ <p style="text-align: center;">(blue colour) (colourless)</p>		
Apparatus/ Instruments	<ol style="list-style-type: none"> 1. 250 mL glass stoppered Erlenmeyer flask. 2. Analytical balance (Readability 0.001 g). 		
Material and Reagents	<ol style="list-style-type: none"> 1. Glacial acetic acid. 2. Chloroform (AR grade). 3. Sodium thiosulphate (> 99% purity). 4. Potassium iodide. 5. Sodium thiosulphate. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Acetic acid-chloroform solution - Mix 3 volumes of acetic acid with 2 volumes of chloroform. 2. Potassium iodide solution, saturated – Dissolve excess KI in freshly boiled water; excess solid must remain; store this solution in dark. Test 		

	<p>daily by adding 0.5 mL to 30 mL CH₃COOH-CHCl₃ (solution a), then add 2 drops of 1% starch solution. If solution turns blue requiring more than 1 drop of 0.1 N sodium thiosulphate to discharge colour, prepare fresh solution.</p> <p>3. Standard sodium thiosulphate solution (0.1 N) - Dissolve approximately 24.8 g of sodium thiosulphate crystals (Na₂S₂O₃.5H₂O) in distilled water and make up to 1000 mL.</p> <p>Standardise this solution by the following procedure: Weigh accurately about 5.0 g of finely powdered potassium dichromate which has been previously dried at 105 ± 2 °C for one hour, dissolve it in distilled water and make up to 1 L. For standardization of sodium thiosulphate, pipette 25 mL of this solution into a 250 mL conical flask. Add 5 mL of concentrated hydrochloric acid and 15 mL of a 10 percent potassium iodide solution. Allow to stand in dark for 5 min and titrate the content with sodium thiosulphate solution using starch as indicator at the end. End point is change of blue colour to green</p> <p>$N = 25W/49.03 V$</p> <p>Where, N = Normality of the sodium thiosulphate W = Weight in g of the potassium dichromate V = Volume in mL of sodium thiosulphate solution required for titration</p> <p>For 0.01 N dilute 0.1 N with freshly boiled and cooled water.</p>
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh 5 ± 0.5 g sample in a 250 mL glass stoppered Erlenmeyer flask, add 30 mL of acetic acid-chloroform solution and swirl to dissolve. 2. Add 0.5 mL of saturated KI solution from Mohr pipette, let it stand with occasional shaking for 1 min, and add 30 mL water. 3. Slowly titrate with 0.1 N sodium thiosulphate solution with vigorous shaking until yellow colour is almost gone. 4. Add about 0.5 mL of starch solution and continue titration by shaking vigorously to release all iodine from chloroform layer until blue just disappears. 5. If less than 0.5 mL 0.1 N Na₂S₂O₃ is used, repeat determination with 0.01 N Na₂S₂O₃. 6. Conduct blank determination (must be less than 0.1 mL 0.1N Na₂S₂O₃); subtract from sample titration.

Calculation with units of expression	Peroxide value (milliequivalents oxygen / kg oil) = $\frac{S \times N \times 1000}{\text{Weight of Sample}}$ Where, S = mL of Na ₂ S ₂ O ₃ (blank corrected) used and N = Normality of Na ₂ S ₂ O ₃
Reference	<ol style="list-style-type: none"> 1. AOAC 965.33. 21st Edn. (2019). Official method Peroxide Value of Oils and Fats. AOAC International, USA. 2. IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Iodine Value in Milk Fat Products

Method No.	FSSAI 01.094:2021	Revision No. & Date	0.0
Scope	This method is applicable for determining the iodine value in milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Carbon tetrachloride: Carbon tetrachloride is a carcinogen. Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Carry out all reaction in a fume hood. 3. Potassium Iodide: It is incompatible with strong oxidizing and reducing agents. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. 		
Principle	<p>The iodine value of oil/fat is the number of grams of iodine absorbed by 100 g of the oil/fat, when determined by using Wij's solution. The iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat. The oil / fat sample taken in carbon-tetrachloride is treated with a known excess of iodine monochloride solution in glacial acetic (Wij's solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine is estimated by titration with sodium thiosulfate solution.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. 250 mL stopper conical flask (Iodine flask). 2. Analytical balance (Readability 0.001 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Potassium dichromate CRM grade. 2. Concentrated hydrochloric acid (Approximately 11.6 N). 3. Glacial acetic acid, free from ethanol. 4. Carbon tetrachloride. 5. Iodine mono-chloride (ICl). 6. Sodium thiosulphate. 7. Mercuric iodide. 8. Potassium iodide (free from potassium iodate). 		

	9. Starch.
Preparation of Reagents	<p>1. Potassium iodide (free from potassium iodate) - 10% solution prepared fresh.</p> <p>2. Starch solution - Mix 5 g of starch and 0.01 g of the mercuric iodide with 30 mL of cold water and slowly pour it with stirring into one litre of boiling water; boil for three min. Allow to cool and decant off the supernatant clear liquid.</p> <p>3. Wij's Iodine monochloride solution - Dissolve 10 mL of iodine monochloride in about 1800 mL of glacial acetic acid and shake vigorously. Pipette 5 mL of Wij's solution, add 10 mL of potassium iodide solution and titrate with 0.1N standard sodium thiosulphate solution using starch as indicator. Adjust the volume of the solution till it is approximately 0.2 N or prepare Wij's iodine solution by dissolving 13 g resublimed iodine in 1 litre acetic acid and pass in dried chlorine (dried through sulphuric acid) until original sodium thiosulphate titre of the solution is not quite doubled (characteristic colour change at the end point indicates proper amount of chlorine. Convenient method is to reserve some amount of original Iodine solution, add slight excess of Cl to bulk of solution and bring to desired titre by re additions of reserved portion). Store in an amber bottle sealed with paraffin until ready for use. Wij's solutions are sensitive to temp, moisture and light. Store in dark at less than 30 °C. Determine I / Cl ratio as follows Iodine Content – Pipette 5 mL Wij Solution into 500 mL Erlenmeyer flask containing 150 mL saturated Cl – water and some glass beads. Shake heat to boiling point and boil briskly 10 min. Cool, add 30 mL sulphuric acid (1 + 49) and 15 mL 15% KI solution and titrate immediately with 0.1 N Na₂S₂O₃.</p> <p>Total Halogen content – Pipette 20 mL Wij's solution into 500 Erlenmeyer flask containing 150 mL recently boiled and cooled water and 15 mL 15% KI solution. Titrate immediately with 0.1 N Na₂S₂O₃.</p> $I / Cl = 2 X / (3B - 2 X)$ <p>where X = mL of 0.1 Na₂S₂O₃ required for I content and B = mL required for total halogen content.</p> <p>I / Cl ratio must be 1.10 ± 0.1</p> <p>4. Standard sodium thiosulphate solution (0.1 N) - Dissolve approximately 24.8 g of sodium thiosulphate crystals (Na₂S₂O₃.5H₂O)</p>


	<p>in distilled water and make up to 1000 mL.</p> <p>Standardise this solution by the following procedure: Weigh accurately about 5.0 g of finely powdered potassium dichromate which has been previously dried at 105 ± 2 °C for one hour, dissolve it in distilled water and make up to 1 L. For standardization of sodium thiosulphate, pipette 25 mL of this solution into a 250 mL conical flask. Add 5 mL of concentrated hydrochloric acid and 15 mL of a 10 percent potassium iodide solution. Allow to stand in dark for 5 min and titrate the content with sodium thiosulphate solution using starch as indicator at the end. End point is change of blue colour to green</p> $N = 25W/49.03 V$ <p>Where, N = Normality of the sodium thiosulphate W = Weight in g of the potassium dichromate V = Volume in mL of sodium thiosulphate solution required for titration</p>
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately 0.53 to 0.65 g of milk fat/ghee, into a 500 mL conical flask with glass stopper, to which 25 mL of carbon tetrachloride has been added; mix the contents well. 2. The weight of the sample shall be such that there is an excess of 50 to 60 percent of Wij's solution over that actually needed. 3. Pipette 25 mL of Wij's solution and replace the glass stopper after wetting with potassium iodine solution. Swirl for proper mixing and keep the flasks in dark for half an hour for non-drying and semi-drying oils and one hour for drying oils. Carry out a blank simultaneously. 4. After standing, add 15 mL of potassium iodide solution, followed by 100 mL of recently boiled and cooled water, rinsing in the stopper also. 5. Titrate liberated iodine with standardized sodium thiosulphate solution, using starch as indicator at the end until the blue colour formed disappears after thorough shaking with the stopper on. 6. Conduct blank determinations in the same manner as test sample but without oil/fat. 7. Slight variations in temperature appreciably affect titre of iodine solution as chloroform has a high coefficient of expansion. It is thus necessary that blanks and determinations are made at the same time.
Calculation with units of expression	$\text{Iodine value} = \frac{12.69 (B - S)N}{W}$

	<p>Where,</p> <p>B = volume in mL of standard sodium thiosulphate solution required for the blank.</p> <p>S = volume in mL of standard sodium thiosulphate solution required for the sample. N = normality of the standard sodium thiosulphate solution.</p> <p>W = weight in g of the sample.</p>
Reference	<p>A.O.A.C. 17th edn, 2000, Official method 920. 159 – Iodine absorption number of oils and fats.</p> <p>IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.</p>
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Saponification Value in Milk Fats

Method No.	FSSAI 01.095:2021	Revision No. & Date	0.0
Scope	This method is applicable for the determination of saponification value in milk fat, <i>ghee</i> , butter oil, anhydrous milk fat and anhydrous butter oil.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Potassium hydroxide is a highly corrosive chemical and contact can irritate and burn the skin and eyes. 2. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 		
Principle	The milk fat sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid. The saponification value is expressed as the number of mg of potassium hydroxide required to saponify 1 g of fat.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. 250 mL capacity conical flask with ground glass joints. 2. 1 m long air condenser, or reflux condenser (65 cm minimum in length) to fit the 250 mL capacity conical flask. 3. Hot water bath or electric hot plate fitted with thermostat. 4. Analytical balance (Readability 0.001 g). 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Aldehyde free alcohol: conforming to IS: 323-1959. 2. Potassium hydroxide. 3. Phenolphthalein indicator. 4. Concentrated hydrochloric acid (Approximately 11.6 N). 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Alcoholic potassium hydroxide solution (0.5 N) - Dissolve 35 to 40 g of potassium hydroxide in 20 mL of distilled water, and add sufficient aldehyde-free alcohol to make up to 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h, then quickly decant the clear supernatant into a suitable, tight container, and standardize the solution to 0.5 ± 0.005 N and keep in a bottle closed tight with a cork or rubber stopper. 2. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein 		

	<p>in 100 mL rectified spirit.</p> <p>3. Standard hydrochloric acid (0.5 N ± 0.005 N): Dilute 4.31 mL of concentrated hydrochloric acid to 100 mL with distilled water. Standardize the prepared solution against 0.5 N sodium carbonate solution.</p>
Sample preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Mix the melted sample thoroughly and weigh about 1.5 to 2.0 g of dry sample into a 250 mL Erlenmeyer flask. 2. Pipette 25 mL of the alcoholic potassium hydroxide solution into the flask; conduct a blank determination along with the sample. 3. Connect the sample flasks and the blank flask with air condensers, keep on the water bath, boil gently but steadily until saponification is complete, as indicated by absence of any oily matter and appearance of clear solution. Clarity may be achieved within 1 h of boiling. 4. After the flask and condenser have cooled wash down the inside of the condenser with about 10 mL of hot ethyl alcohol neutral to phenolphthalein. 5. Titrate the excess potassium hydroxide with standardised 0.5 N hydrochloric acid, using about 1.0 mL phenolphthalein indicator.
Calculation with units of expression	$\text{Saponification Value} = \frac{56.1 (B - S)N}{W}$ <p>Where,</p> <p>B = Volume in mL of standard hydrochloric acid required for the blank.</p> <p>S = Volume in mL of standard hydrochloric acid required for the sample</p> <p>N = Normality of the standard hydrochloric acid</p> <p>W = Weight in g of the oil/fat taken for the test</p> <p>56.1 = molecular weight of potassium hydroxide</p>
Reference	<ol style="list-style-type: none"> 1. A.O.A.C 17th edn, 2000, Official method 920.160 Saponification number of oils and fats / 2. IS 3508: 1966 (Reaffirmed year 2018). Methods of sampling and test for Ghee (Butterfat). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Detection of Adulteration of Milk Fat Products with Vegetable Oils by Reversed Phase- High Performance Liquid Chromatography (RP- HPLC)		
Method No.	FSSAI 01.096:2021	Revision No. & Date	0.0
Scope	<p>This method is applicable for the detection of adulteration ghee with vegetable oils by RP-HPLC. The method is applicable for the detection of the soybean oil, coconut oil, sunflower oil, groundnut oil and designer vegetable oil in ghee.</p>		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Potassium hydroxide: It is highly corrosive. It reacts violently with strong acids. While making solutions add pellets to water and not vice versa. 2. Chloroform: Decomposes slowly on exposure to light and on exposure to air it releases toxic and corrosive gases/vapours (phosgene, chlorine, hydrogen chloride). Perform all operations under a fume hood. 3. Methanol: Highly flammable; keep away from heat, sparks and open flame. Perform all operations under a fume hood. 4. n-hexane: Avoid storage near extreme heat, ignition sources or open flame. Use respiratory protective device against the effect of fumes/aerosols. Perform all operations under an effective fume removal device. 5. Acetonitrile: Do not inhale substance/mixture. Avoid generation of vapours/aerosols. Keep away from open flames, hot surfaces and sources of ignition. Keep container tightly closed in a dry and well-ventilated place (+5 °C to +30 °C). 		
Principle	<p>The RP-HPLC method is based on the detection of cholesterol and β-sitosterol as markers in the unsaponifiable matter (USM). β- Sitosterol, a plant sterol serves as an indicator for the adulteration in ghee by vegetable oils</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Beakers (100 mL, 1000 mL). 2. Volumetric flasks (10 mL, 100 mL, 200 mL). 3. Screw capped test tubes (50 mL). 4. Micro centrifuge stands (2 No.). 5. Adjustable micro pipettes (1 mL and 10 mL). 6. Millipore filter paper Nylon (0.22 μm). 7. Analytical balance (Readability 0.001 g). 8. Shaking water bath. 9. Vortex mixer. 10. Centrifuge. 		

	<p>11. HPLC equipped with Photo Diode Array (PDA) detector, pumps, auto sampler, column oven and degasser.</p> <p>12. Column: Reversed phase C-18, 4.6 × 250 mm ID, 5μ 120 Å particle size</p> <p>13. Micro concentrator.</p>
Materials and Reagents	<p>1. β- sitosterol- Purity.</p> <p>2. Stigmasterol- Purity.</p> <p>3. Methanolic potassium hydroxide.</p> <p>4. n-Hexane (HPLC grade).</p> <p>5. Chloroform.</p> <p>6. Methanol.</p> <p>7. Cholesterol.</p> <p>8. Acetonitrile (HPLC grade).</p> <p>9. Isopropanol (HPLC grade).</p>
Preparation of reagents	<p>Preparation of stock solution of standards</p> <p>1) Stock solution A (Cholesterol): Prepare 1 mg/mL of stock solution in chloroform.</p> <p>2) Stock solution B (Stigmasterol): Prepare 1 mg/mL of stock solution in chloroform.</p> <p>3) Stock solution C (β-sitosterol): Prepare 1 mg/mL of stock solution in chloroform.</p> <p>4) Prepare diluted standards up to 1 μg/mL of individual and mixture of (1 to 3) in methanol.</p>
Sample Preparation	Refer method FSSAI 01.084:2021 for sample preparation.
Method of analysis	<p>Preparation of unsaponifiable matter (USM)</p> <p>1. Weigh 1 g of fat in a screw capped test tube.</p> <p>2. Add 25 mL of 5% methanolic potassium hydroxide.</p> <p>3. Keep the tubes in water bath maintained at 90 °C for 50 min with vigorous shaking at regular intervals.</p> <p>4. Take out the tubes from water bath and add 5 mL water and 15 mL hexane.</p> <p>5. Vortex the mixture for 1min.</p> <p>6. Centrifuge at 1700 g (around 3000 rpm) for 5 min.</p> <p>7. Pipette out the upper hexane layer completely and dry (preferably in micro concentrator) to USM.</p> <p>8. Dissolve the dried USM in 300 μl of chloroform and add 500 μl methanol, vortex and mix.</p> <p>9. Filter the above extract through 0.22 μm nylon filter paper.</p> <p>HPLC analysis</p>

Perform the HPLC analysis on the material obtained above after filtration. HPLC conditions are mentioned below Run the sample (obtained after filtration from 0.22 μm nylon filter paper), reference standards of cholesterol, stigmasterol and β - sitosterol also on HPLC.

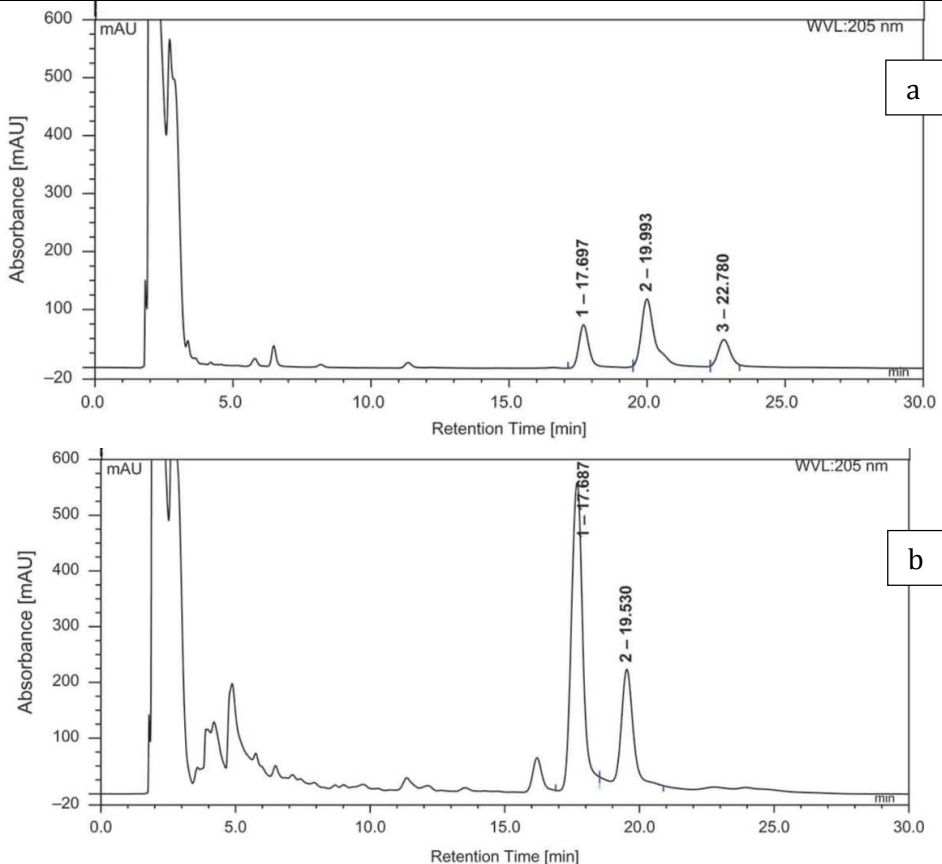
HPLC Condition

Column	C-18, 4.6 mm (i.d.) \times 250 mm, 5 μm
Column Oven Temp	30 $^{\circ}\text{C}$
Flow rate	1.5 mL/min
PDA wavelength	205 nm
Injection volume	20 μl
Run time	35 min
Washing solution	Acetonitrile: Isopropanol (9:1)


Mobile Phase: Acetonitrile: Isopropanol (9:1), filtered and degassed
The RP-HPLC method resolves cholesterol, stigmasterol and β - sitosterol at Retention Time (RT values) of 22.8 \pm 0.2, 25.5 \pm 0.2, 29.05 \pm 0.2 min under prescribed conditions of the method.


Peak identification and Confirmation


1. Identify the peaks in the sample by comparing the retention time with that of reference standards.
2. Limit of Detection (LOD) would vary with the type of oil adulterated.
3. Method is capable of detecting coconut oil at 5%, refined soybean oil at 1%, groundnut oil at 2% and sunflower oil at 1%.
4. The detection and reporting of any result below than 1% should be done cautiously.

<p>Calculation with units of expression</p>	 <p>Figure: a) HPLC chromatogram of standard cholesterol (Retention time: 17.697 min), Campasterol and stigmasterol (Retention time: 19.933 min), β-sitosterol (Retention time: 22.780 min). b) HPLC chromatogram of unsaponifiable matter of pure cow ghee (Rani et al., 2016)</p>
<p>Inference (Qualitative analysis)</p>	<ol style="list-style-type: none"> 1. In pure/genuine ghee sample the peak of β- sitosterol must be absent 2. The detection of β- sitosterol peak denotes the adulteration of ghee with vegetable fat.
<p>Reference</p>	<ol style="list-style-type: none"> 1. FSSAI Order. (2019). Method for detection of adulteration in ghee (clarified milk fat) with vegetable oils. File No. 1-90/FSSAI/SP (MS&A)/2009 dated 25th March, 2018. Food Safety Standards Authority of India, New Delhi. 2. Rani, A., Sharma, V., Arora, S., & Ghai, D. L. (2016). Comparison of rapid reversed phase high-performance liquid chromatography (RP-HPLC) method with rapid reversed phase thin layer chromatography method for detecting vegetable oils in ghee (clarified milk fat). International Journal of Food Properties, 19(5), 1154-1162.
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>

L. CHAKKA AND SHRIKHAND

 Preparation of Sample of Chakka and Shrikhand	
Method No.	FSSAI 01.097:2021 Revision No. & Date 0.0
Scope	This method is used for the preparation of <i>chakka</i> and <i>shrikhand</i> for analysis.
Apparatus/Instruments	Mortar and pestle
Sample preparation	<ol style="list-style-type: none">1. To get a representative sample, particularly in case of a thick-set product, it may be necessary to pour out the whole of the contents in a mortar and mix it thoroughly until the mass is homogeneous.2. Aliquots are weighed out from this well-mixed sample.
Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Solids in <i>Chakka</i> and <i>Shrikhand</i>		
Method No.	FSSAI 01.098:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of total solids in <i>chakka</i> and <i>shrikhand</i> .		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	Total solids is determined by drying the product in hot air oven at 102 ± 2 °C to constant mass.		
Apparatus/Instruments	Refer method prescribed for analysis of total solids in condensed/evaporated milk (FSSAI 01.052:2021).		
Materials and reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.097:2021 for sample preparation.		
Method of analysis	Refer method prescribed for analysis of total solids in condensed/evaporated milk (FSSAI 01.052:2021).		
Calculation with units of expression	$\text{Total Solids Content (\%)} = \frac{M_2 - M}{M_1 - M} \times 100$ <p>Where,</p> <p>M is the mass in g of empty dish along with lid; M₁ is the initial mass in g of dish with lid and test portion M₂ is mass in g of dish with lid and dried test portion Round the value obtained to nearest 0.01 % (m/m)</p> <p>Note: BIS/ISO method does not recommend use of sand. However, use of sand help in even spreading of samples.</p>		
Reference	IS 11622: 1986. Method for determination of total solids content in condensed milk. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Fat (on dry matter basis) in <i>Chakka</i> and <i>Shrikhand</i>		
Method No.	FSSAI 01.099:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of fat on dry matter basis in <i>chakka</i> and <i>shrikhand</i> .		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	Refer principle stated for analysis of fat in condensed milk (FSSAI 01.050:2021).		
Apparatus/Instruments	Refer requirement stated for analysis of fat in condensed milk (FSSAI 01.050:2021).		
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.097:2021 for sample preparation.		
Method of analysis	<p>Rose-Gottlieb Method Refer method stated for analysis of fat in condensed milk (FSSAI 01.050:2021).</p> <p>Determination of Total Solids Refer method stated for analysis of total solids in <i>chakka</i> and <i>shrikhand</i> (FSSAI 01.098:2021).</p>		
Calculation with units of expression	$\text{Fat \% (m/m)} = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p>		


	<p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p> $\text{Fat \% (on dry matter basis)} = \frac{\text{Fat (\%)}}{\text{Total Solids (\%)}} \times 100$
Reference	<ol style="list-style-type: none"> 1. IS 9352: 1980 (Reaffirmed year 2019). Specifications for chakka and shrikhand. Bureau of Indian Standards, New Delhi. 2. IS 1166: 1986 (Reaffirmed year 2018). Specifications for condensed milk, partly skimmed condensed milk (Second Revision). Bureau of Indian Standards, New Delhi. 3. Pearson's Composition and analysis of foods 9th edn, 1991 page 604.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Milk Protein (on dry matter basis) in <i>Chakka</i> and <i>Shrikhand</i>		
Method No.	FSSAI 01.100:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of milk protein in <i>chakka</i> and <i>shrikhand</i> and expressed on dry matter basis		
Introduction/ Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk and milk products (FSSAI 01.025/026:2021).		
Apparatus			
Material and Reagents	The total solids in the product is calculated separately and the % protein is calculated on dry matter basis		
Preparation of Reagents			
Sample preparation	Refer method FSSAI 01.097:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 1-2 g of the prepared sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube. 		

	<p>2. Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021).</p> <p>Determination of Total Solids Refer method stated for analysis of total solids in chakka and shrikhand (FSSAI 01.098:2021).</p>
Calculation with units of expression	<p>Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021)</p> $\text{Protein \% (on dry matter basis)} = \frac{\text{Protein (\%)}}{\text{Total Solids (\%)}} \times 100$
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Titratable Acidity of <i>Chakka</i> and <i>Shrikhand</i>		
Method No.	FSSAI 01.101:2021	Revision No. & Date	0.0
Scope	This method is applicable for the determination of titratable acidity in <i>chakka</i> and <i>shrikhand</i> .		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	A known quantity of sample is neutralized with standardized sodium hydroxide solution with phenolphthalein indicator. The amount of sodium hydroxide required is a function of the amount of natural buffering substances present in the product, and of developed or added acid or alkaline substances.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.01 g). 2. Burette, 50 mL. 3. Porcelain dishes, white hemispherical of approximately 60 ml capacity. 4. Stirring rods, of glass, flattened at one end. 5. Pipettes, 10 mL and 1 mL. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Phenolphthalein indicator. 3. Ethanol. 		
Preparation of reagents	<ol style="list-style-type: none"> 1. Standard sodium hydroxide solution – 0.1 N. 2. Phenolphthalein Indicator – Dissolve 1.0 g of phenolphthalein in 100 mL of 95% ethanol. Add 0.1 N Sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 mL. 		
Sample preparation	Refer method FSSAI 01.097:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 10 g of the sample into each of the two porcelain dishes. 2. Add 30 ml of lukewarm water to each dish and stir with the flat end of a glass rod until a fine paste is obtained. Use the contents of one dish as a blank for comparison of colour. 		

	<p>3. Add 1 ml of phenolphthalein indicator solution in the other dish, shake well and titrate against standard sodium hydroxide solution. Stir vigorously throughout.</p> <p>Note: The time taken for the complete titration shall not exceed 20 seconds. The persistence of slight pinkish tinge for 30 seconds. The titration shall be preferably made in north light or under illumination from a day light lamp.</p>
Calculation with units of expression	<p>Titratable acidity (as lactic acid) percent by mass = $\frac{9AN}{w}$</p> <p>Where,</p> <p>A = Volume of standard Sodium hydroxide required for titration</p> <p>N = Normality of standard Sodium hydroxide solution</p> <p>w = weight of the sample taken for test in g</p>
Reference	IS 1166: 1986 (Reaffirmed year 2018). Specifications for condensed milk, partly skimmed condensed milk (Second Revision). Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Total Ash (on dry matter basis) in <i>Chakka</i> and <i>Shrikhand</i>		
Method No.	FSSAI 01.102:2021	Revision No. & Date	0.0
Scope	This method is applicable for the determination of total ash on dry matter basis in <i>chakka</i> and <i>shrikhand</i> .		
Caution	Follow all safety procedures and wear laboratory apron and safety goggles. <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot crucibles. 2. Hot crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	Sample is kept at 550 ± 25 °C in muffle furnace so that all the organic matter will be burn left behind the inorganic matter that is ash. Total solids are estimated separately in the sample (Refer method FSSAI 01.098:2021). Then, total ash (on dry matter basis) is calucalted in the sample.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Platinum or silica crucible, about 70 mm diameter and 25 to 50 mm deep. 2. Muffle furnace, capable of being controlled at 550 ± 25 °C. 3. Desiccator containing an efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 4. Steam bath 5. Safety tongs having long arms. 6. Bunsen burner or electric hot plate. 7. Analytical balance (Readability 0.0001g) 8. Thermal protection gloves, capable to resist temperature up to 550-600 °C 9. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C 		
Sample preparation	Refer method FSSAI 01.097:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 3 g of the sample in a crucible, previously dried in a hot air oven and weighed. 2. Evaporte the moisture in the sampel by keeping the dish on steam bath. 3. Heat the crucible gently on a burner or hot plate at first and then strongly in a muffle furnace at 550 ± 25 °C till grey ash is obtained. 4. Cool the crucible in a desiccator and weigh it. 5. Heat the crucible again at 550 ± 25 °C for 30 min. 6. Cool the crucible in a desiccator and weigh. 7. Repeat this process of heating for 30 min, cooling and weighing until the difference between two successive weighing is less than 1 mg. 8. Record the lowest mass. 		


	<p>Determination of Total Solids</p> <p>Refer method stated for analysis of total solids in chakka and shrikhand FSSAI 01.098:2021.</p>
Calculation with units of expression	<p>Total ash (% by mass) = $\frac{M_2 - M}{M_1 - M} \times 100$</p> <p>Where,</p> <p>M = mass in g, of the empty crucible</p> <p>M₁ = mass in g, of the crucible with the material taken for the test; and</p> <p>M₂ = mass in g, of the crucible with ash;</p> <p style="text-align: center;">Total ash % (on dry matter basis) = $\frac{\text{Total ash (\%)}}{\text{Total Solids (\%)}} \times 100$</p>
Reference	IS 1165: 2002 (Reaffirmed year 2018). Milk Powder – Specifications. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis


Determination of Sucrose Content in *Shrikhand*

Method No.	FSSAI 01.103:2021	Revision No. & Date	0.0
Scope	This method is for the determination of sucrose content in <i>shrikhand</i> . Sucrose content can be determined by any of the following methods: Volumetric method (Lane - Eynon) and Polarimetric method.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide solution: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ammonia solution: It reacts violently with halogens (such as fluorine, chlorine, bromine), acids (such as hydrochloric acid, hydrogen bromide, hydrogen fluoride). It is incompatible with cyanide and oxidizing agents. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 		
Principle	Refer method prescribed for analysis of sucrose content in condensed/evaporated milk (FSSAI 01.051:2021).		
Lane-Eynon (Volumetric) Method,			
Polarimetric Method			
Apparatus/Instruments			
Materials and Reagents			
Preparation of Reagents			
Sample preparation	Refer method FSSAI 01.097:2021 for sample preparation.		
Method of analysis	Refer method prescribed for analysis of sucrose content in condensed milk (FSSAI 01.051:2021).		

Calculation with units of expression	Refer method prescribed for analysis of sucrose content in condensed milk (FSSAI 01.051:2021).
Reference	<ol style="list-style-type: none"> 1. IS 4079 – 1967 (Reaffirmed year 2016). Specification for canned rasogolla. Bureau of Indian Standards, New Delhi. 2. IS 11764 – 2005 (Reaffirmed year 2019). Method for determination of sucrose content by polarimetric method in condensed milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

M. WHEY POWDER

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Preparation of Sample of Whey Powder and Acid Whey Powder		
Method No.	FSSAI 01.104:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of whey powder.		
Sample preparation	<ol style="list-style-type: none">1. Make the sample homogeneous either by mixing or shaking alternately, and rolling and inverting the container.2. Avoid excessive temperature and humidity when opening the sample container to prevent absorption of moisture.		
Reference	FSSAI manual of methods of analysis of foods: milk and milk products. (2016). Food Safety Standards Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		


 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of Moisture in Whey Powder and Acid Whey Powder		
Method No.	FSSAI 01.105:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of moisture in all whey powders.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The sample is dried to constant weight at 102 ± 2 °C and the loss in weight reported as moisture.		
Apparatus/Instruments	Refer method prescribed for analysis of moisture content in milk powder (FSSAI 01.055:2021).		
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.104:2021 for sample preparation.		
Method of analysis	Refer method prescribed for analysis of moisture content in milk powder (FSSAI 01.055:2021).		
Calculation with units of expression			
Reference	<ol style="list-style-type: none"> 1. IS 16072: 2012 (Reaffirmed year 2018). Determination of moisture content in milk powder and similar products, Bureau of Indian Standards: India. 2. ISO 5537/IDF 026:2004 - Dried milk - Determination of moisture content (Reference method). International Organization for Standardization, Geneva. 3. F.A.O Manuals of Food quality Control 14/ 8 page 30). 		
Approved by	Scientific Panel on Methods of Sampling and Analysis		


Determination of Fat in Whey Powder and Acid Whey Powder


Method No.	FSSAI 01.106:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of fat in all whey powders.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	Refer method prescribed for analysis of fat content in milk powder (FSSAI 01.056:2021).		
Apparatus/Instruments			
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.104:2021 for sample preparation.		
Method of analysis	Refer method prescribed for analysis of fat content in milk powder (FSSAI 01.056:2021).		
Calculation with units of expression			
Reference	IS 11721 – 2013 (Reaffirmed year 2018). Method of sampling and test for dried milk sample. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 Determination of Milk Protein in Whey Powder and Acid Whey Powder	
Method No.	FSSAI 01.107:2021 Revision No. & Date 0.0
Scope	This method is used for the determination of protein in all whey powders.
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalies as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation.
Principle	Refer the method for determination of protein by Kjeldahl method in milk and milk products (FSSAI 01.025/026:2021).
Apparatus/Instruments	
Materials and Reagents	
Preparation of Reagents	
Sample preparation	Refer method FSSAI 01.104:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 1-1.3 g of the prepared whey powder sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube.

	2. Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021).
Calculation with units of expression	Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021)
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Total Ash (on dry matter basis) in Whey Powder and Acid Whey Powder		
Method No.	FSSAI 01.108:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of total ash (on dry matter basis) in whey powder and acid whey powder.		
Caution	Follow all safety procedures and wear laboratory apron and safety goggles. <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot crucibles. 2. Hot crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The sample is kept at 550 ± 20 °C in a muffle furnace so that all the organic matter will be burnt. The leftover inorganic matter is the total ash.		
Apparatus/Instruments	Refer method prescribed for analysis of total ash in milk powder (FSSAI 01.063:2021).		
Materials and Reagents	The total solids in the product are calculated and the % total ash is calculated on dry matter basis.		
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.104:2021 for sample preparation.		
Method of analysis	<p>Determination of ash Refer method prescribed for analysis of total ash in milk powder (FSSAI 01.063:2021).</p> <p>Determination of moisture Refer method prescribed for analysis of moisture in whey powder (FSSAI 01.105:2021).</p>		
Calculation with units of expression	Total ash (on dry basis), % by mass = $\frac{M_2 - M}{(100 - M_0) \times (M_1 - M)} \times 100$ <p>Where,</p> <p>M_0 = moisture, % by mass, calculated as per the method for dried milk</p> <p>M = mass in g, of the empty crucible;</p> <p>M_1 = mass in g, of the crucible with the material taken for the test; and</p> <p>M_2 = mass in g, of the crucible with ash;</p>		
Reference	IS 1165 – 2002 (Reaffirmed year 2018). Milk Powder – Specifications. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of pH in Whey Powder and Acid Whey Powder		
Method No.	FSSAI 01.109:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of pH in whey powder and acid whey powder.		
Caution	Wear laboratory apron, shoes, safety goggles and mask while working in laboratory.		
Principle	Refer method prescribed for analysis of pH in Casein / Caseinate (FSSAI 01.119:2021)		
Apparatus/Instruments			
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.104:2021 for sample preparation.		
Method of analysis	Refer method prescribed for analysis of pH in Casein / Caseinate (FSSAI 01.119:2021).		
Calculation with units of expression	Refer method prescribed for analysis of pH in Casein / Caseinate (FSSAI 01.119:2021).		
Reference	IS 11918: 2017. Method for determination of pH in caseins and caseinates (Reference Method). Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Lactose in Whey Powder and Acid Whey Powder		
Method No.	FSSAI 01.110:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of lactose in whey powder and acid whey powder by Lane – Eynon method.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 2. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 3. Copper sulphate: Non-combustible, substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. 4. Glacial acetic acid: Use with adequate ventilation. Keep away from heat. Do not breathe gas/fumes/ vapor/spray. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, alkalis etc. 		
Principle	Lane and Eynon method is based on the principle of reduction of Fehling’s solution by reducing sugars (Lactose). Fehling’s solution is a mixture of copper sulphate and alkaline Rochelle salt (sodium potassium tartarate). Rochelle salt complexes with the cupric hydroxide formed in alkaline solution and prevent it from precipitation. Lactose reduces the complexed cupric hydroxide to red, insoluble cuprous oxide under the experimental conditions. An oxidation-reduction indicator, usually methylene blue, detects the end point of the reaction.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.01 g). 2. Hot plate. 3. Burette (50 mL cap.) with an off-set tip. 4. Volumetric flask, 250 mL. 5. Pipette, 5 mL and 25 mL. 6. Conical flask, 250 mL. 		

	<ol style="list-style-type: none"> 7. Funnel (small). 8. Whatman No. 1 filter circles.
Materials and Reagents	<ol style="list-style-type: none"> 1. Copper sulphate. 2. Concentrated sulphuric acid (Approximately 36.8 N). 3. Rochelle salt (sodium potassium tartrate). 4. Sodium hydroxide. 5. Acetic acid. 6. Lactose. 7. Methylene blue.
Preparation of reagents	<p>Dilute sulphuric acid (1 N): Dilute 27.17 mL of concentrated sulphuric acid to 1000 mL with distilled water.</p> <p>Fehling's Solution: It is freshly prepared by mixing equal volumes of the following two solutions, termed as Fehling's A and Fehling's B.</p> <ol style="list-style-type: none"> 1. Fehling's A: Dissolve 34.639 g of copper sulphate in sufficient distilled water, add a few drops of 1 N sulphuric acid to prevent formation of cloudy appearance or turbidity (use a minimum quantity of acid). Dilute the solution with distilled water to 500 mL. If necessary, filter through prepared asbestos, on Gooch crucible. 2. Fehling's B: Dissolve 173 g Rochelle salt in distilled water. Add to it 50 g of sodium hydroxide and dilute the mixture to 500 mL with distilled water. Allow to stand in a rubber or polyethene stoppered container for two days and filter through prepared asbestos. 3. Acetic acid solution: 10% (w/v; aqueous) solution. 4. Methylene blue indicator: 1% (w/v; aqueous) solution. 5. Standard lactose solution: 0.5% (w/v; aqueous) solution: Weigh accurately 5 g of this dried lactose and dissolve in freshly boiled and cooled distilled water. Transfer the solution quantitatively to 1 L volumetric flask and dilute up to mark with distilled water. Mix well and store it in a cool place. One milileter of this solution contains 5 mg (0.005 g) lactose.
Sample preparation	Refer method FSSAI 01.104:2021 for sample preparation.
Method of analysis	<p>Preparation of sample filtrate</p> <ol style="list-style-type: none"> 1. Take 2-2.5 g of prepared sample into a 100 mL volumetric flask. 2. Add 30 to 40 mL distilled water and the content of the flask are warmed to 40 to 45 °C, immediately add 1.5 mL acetic acid (10%). Mix well and make the final volume with distilled water up to mark and mix again. Keep the flask undisturbed for 30 min. 3. After 30 min, filter the content of the flask through a Whatman filter paper Grade 42. Discard the first few milileter of the filtrate and collect the rest in a clean dry Erlenmeyer flask fitted with stopper.

B. Standardization of Fehling's solution

Preliminary titration

1. Pipette 5 mL of Fehling's solution A and 5 mL of Fehling's solution B using two separate pipettes in a 250 mL Erlenmeyer flask.
2. Fill up a burette with the standard lactose solution and connect the burette end with an offset tube to keep the burette tube out of steam.
3. Heat the content of the flask to boiling over burner or heater and maintain moderate boiling for 2 min. To prevent bumping add some inert boiling chips. Add 3 to 4 drops of methylene blue indicator without removing from the flame.
4. Titrate the content of the flask against standard lactose solution from the burette until the blue colour disappears and the bright brick-red colour of precipitated Cu_2O appears (at the end point the Cu_2O suddenly settles down giving a clear supernatant).
5. Note the volume of lactose solution required for the standardization of Fehling's solution

Final titration

1. After this preliminary titration, further titration or titrations should be carried out, adding practically the whole of the standard lactose solution volume (one mL less than required as observed in first titration) required before commencing the heating and continuing the titration as before.
2. The titration must be completed within 3 min from the commencement of boiling. Let V_1 mL be the titre for this experiment.

Note: Carefully note the first disappearance of blue colour. Once missed, it is difficult to ascertain the end point. Maintain the boiling at a uniform rate during the titration.

C. Determination of lactose in sample


Here also the titration is taken up to two stages. In the first stage, an approximate estimation is made, while in the second stage more accurate result is obtained.


Preliminary titration

1. Pipette 5 mL of Fehling's solution A and 5 mL of Fehling's solution B using two separate pipettes in a 250 mL Erlenmeyer flask.
2. Fill up a burette with the prepared milk filtrate and connect the burette end with an offset tube to keep the burette tube out of steam.
3. Add 10 mL of the milk filtrate from the burette into flask containing Fehling's solution and heat to boiling on wire gauge over burner.
4. Boil for about 15 secs and rapidly add further amount of lactose filtrate until only faint perceptible blue colour remains. Add 3 - 4 drops of


	<p>methylene blue indicator and complete the titration to the first disappearance of blue colour by adding lactose filtrate drop-wise.</p> <p>Final Titration</p> <p>Repeat the experiment of Stage 1, but add almost the entire prepared milk filtrate (one milileter less than required as observed in first titration) required to reduce all copper, as determined by Stage 1, and complete the titration as described under standardization of Fehling's solution. Let V₂ mL be the titer for this experiment.</p>
Calculation with units of expression	$\text{Lactose (\% by mass)} = \frac{0.005 \times V_1 \times 100}{V_2 \times W} \times 100$ <p>Where</p> <p>V₁ = volume in mL of standard lactose solution taken to reduce 10 mL of Fehling's solution; and</p> <p>V₂ = volume in mL of prepared milk filtrate taken to reduce 10 mL of Fehling's solution.</p> <p>W = Mass of sample taken.</p>
Reference	Sharma, R., Mann, B., Lal, D., Rajput, Y.S. & Lata K. (2013). Laboratory Manual on Milk Carbohydrates, Minerals and Water-Soluble vitamins, Published by NDRI.
Approved by	Scientific Panel on Methods of Sampling and Analysis

N. EDIBLE CASEIN PRODUCTS

 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	<h3>Preparation of Sample of Edible Casein Products</h3>		
Method No.	FSSAI 01.111:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of sample of edible acid casein, edible rennet casein and edible caseinates.		
Apparatus/Instruments	Sieve (500 µm)		
Sample preparation	<ol style="list-style-type: none"> 1. Thoroughly mix the sample by repeatedly shaking and inverting the container. 2. Transfer about 50 g of the thoroughly mixed sample to the test sieve (500 µm). 3. If the 50 g portion directly passes or almost completely passes through the sieve, use it for analysis, otherwise, grind the 50 g portion, using the grinding device until it passes through the sieve. 4. Immediately transfer the entire sieved sample to an air-tight container of sufficient capacity and mix thoroughly by repeatedly shaking and inverting. 5. During these operations take precautions to avoid any change in the water content of the product. After the test sample has been prepared, the analysis should be carried out as soon as possible. 		
Reference	IS 11920: 2012 (Reaffirmed year 2018). Caseins ad Caseinates – Determination of moisture content (reference method). Bureau of Indian Standards: India.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <i>Inspiring Trust, Assuring Safe & Nutritious Food</i> Ministry of Health and Family Welfare, Government of India</p>	Determination of Moisture in Edible Casein Products		
Method No.	FSSAI 01.112:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of moisture in edible acid casein, edible rennet casein and edible caseinates.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	The sample is dried to constant weight at 102 ± 2 °C and the loss in weight reported as moisture. The method described here has been followed from IS 16072:2012.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Flat-bottom moisture dishes with cover made of stainless steel, nickel or aluminium having approximately 50 mm diameter and 25 mm depth. The dishes shall have lids which fit well and can readily be removed. 2. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 3. Desiccator containing an efficient desiccant. (for example freshly dried silica gel with a hygroscopic indicator). 		
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Uncover a dish and place the dish and its lid in a hot air oven at 102 ± 2 °C for 1 h. Place the lid on the dish, transfer the covered dish from the hot air oven to the desiccator. Allow it to cool to 25 ± 3 °C and weigh it. 2. Put approximately 2 g of the caseinate sample in the dish, cover the dish with the lid and weigh the covered dish accurately and quickly. 3. Uncover the dish and put it with its lid in the hot air oven maintained at 102 ± 2 °C for 3 h. 4. Replace the lid, transfer the covered dish to the desiccator, allow it to cool to 25 ± 3 °C (for approximately 30-45 min) and weigh it accurately and quickly. 5. Heat the uncovered dish and lid in the hot air oven at 102 ± 2 °C for further 1 h; replace the lid and allow the covered dish to cool to 25 ± 3 °C in the desiccator and weigh it. 6. Repeat the process of drying, cooling and weighing, until the successive weighing do not differ by more than 0.5 mg. It is usually found that drying is complete after the first 2 h. 		

<p>Calculation with units of expression</p>	<p style="text-align: center;">Moisture % by mass = $\frac{M_1 - M_2}{M_1 - M} \times 100$</p> <p>Where, M is mass in g, of the empty dish with lid; M₁ is initial mass in g of the dish with lid and test portion; M₂ is the mass in g of the dish with lid and dried test portion Express the results to the nearest 0.01% (m/m).</p> <p>Note: Commercial automatic moisture determination apparatus can also be used for determination of moisture in milk powder. ISO (ISO 5537:2004) method advocates the use of drying oven set at 87 °C (for 5 h) and use of copper tubes.</p>
<p>Reference</p>	<ol style="list-style-type: none"> 1. IS 11920: 2012 (Reaffirmed year 2018). Caseins ad Caseinates – Determination of moisture content (reference method). Bureau of Indian Standards: India. 2. ISO 5537/IDF 026:2004. Dried milk - Determination of moisture content (Reference method). International Organization for Standardization, Geneva.
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	<h3>Determination of Fat in Edible Casein Products</h3>		
Method No.	FSSAI 01.113:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of fat in edible acid casein, edible rennet casein and edible caseinates.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Light petroleum ether: Avoid storage near extreme heat, ignition sources or open flame. Use respiratory protective device against the effect of fumes/aerosols. Perform all operations under an effective fume removal device. 		
Principle	<p>In this method, a test portion is digested with hydrochloric acid followed by addition of ethanol. The acid-ethanolic solution is subsequently extracted with diethyl ether and light petroleum, followed by removal of solvents by distillation or evaporation and thereafter the determination of mass of the substances extracted which are soluble in light petroleum. The following method is as per IDF guidelines.</p>		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Centrifuge, for the centrifugation of fat-extraction flasks or tubes at 500 to 600 rpm to produce a gravitational field of 80 to 90 g at the outer end of the flasks or tubes (the use of the centrifuge is optional but recommended). 3. Distillation or evaporation apparatus to enable the solvents and ethanol to be distilled from the fat-collecting flasks or to be evaporated from beakers and dishes at a temperature not exceeding 100 °C. 		

	<ol style="list-style-type: none"> 4. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 5. Boiling water bath or hot plate. 6. Mojonnier type fat-extraction tube; the flask shall be provided with good quality bark corks or stoppers of other material for e.g. silicon rubber or polytetrafluoroethylene (PTFE) unaffected by the reagents used. 7. Fat-collecting vessels for e.g., flat bottom flask of capacity 125 to 250 mL. 8. Measuring cylinder of capacities 5 and 25 mL. 9. Pipettes graduated of capacity 10 mL.
Materials and Reagents	<ol style="list-style-type: none"> 1. Hydrochloric acid (Approximately 11.6 N). 2. Ethanol or ethanol denatured by methanol at least 94% (w/v). 3. Congo red. 4. Diethyl ether, free from peroxides containing no, or not more than 2 g/kg of antioxidants. 5. Light petroleum, having any boiling range between 30 and 60 °C.
Preparation of reagents	<ol style="list-style-type: none"> 1. Hydrochloric acid solution of ρ_{20} approximately 1.125 g/mL. Dilute 675 mL of concentrated hydrochloric acid, ρ_{20} 1.18 g/mL, to 1000 mL with water. 2. Congo red solution: Dissolve 1 g of congo red in water and dilute to 100 mL 3. Mixed solvents; prepared shortly before use by mixing equal volumes of diethyl ether and the light petroleum ether.
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Mix and weigh immediately 2 to 3 g of test sample to the nearest 1 mg, directly or by difference, into a fat-extraction flask, or into 100 mL beaker or flask. 2. Add 7.5 to 10 mL of hydrochloric acid solution so as to wash the test portion into the small bulb of the extraction flask (as per BIS method, take 5 g of the sample and 10 mL of hydrochloric acid solution. 3. Carry out a blank test simultaneously with the determination, using the same procedure and same reagents, but omitting the test portion. 4. Heat by gently moving the vessel in a boiling water-bath or on a hot plate, until all the particles are entirely dissolved. Allow the vessel to stand for 20 to 60 min in the boiling water bath, shaking occasionally during the initial 15 min, or keep it gently boiling over the flame or on to the hotplate for 10 min; cool in running water. 5. Proceed for the extraction fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.023:2021).

Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying</p> <p>M₂ is the mass, in g, of the empty fat-collecting flask</p> <p>M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying</p> <p>M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test</p> <p>w is the weight of the sample in g</p>
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Protein in Edible Casein Products


Method No.	FSSAI 01.114:2021	Revision No. & Date	0.0
Scope	This method is applicable for the determination of protein in edible acid casein, edible rennet casein and edible caseinates.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk and milk products (FSSAI 01.026:2021)		
Apparatus/Instruments			
Materials and Reagents			
Preparation of reagents			
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 1-2 g of the prepared sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube. 		

	<p>2. Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021).</p> <p>Determination of moisture Refer method for determination of moisture in edible casein products or FSSAI 01.112:2021.</p>
Calculation with units of expression	<p>Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021).</p> <p>Total Solids (%) = 100 - moisture (%)</p> $\text{Protein \% (on dry matter basis)} = \frac{\text{Protein (\%)}}{\text{Total Solids (\%)}} \times 100$
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Casein in Protein in Edible Casein Products		
Method No.	FSSAI 01.115:2021	Revision No. & Date	0.0
Scope	This method is for the determination of casein in protein in edible acid casein, edible rennet casein and edible caseinates.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Glacial acetic acid: Use with adequate ventilation. Keep away from heat. Do not breathe gas/fumes/ vapor/spray. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, alkalis. 2. Sodium acetate: Combustion products may include carbon oxides or other toxic vapours. Thermal decomposition can lead to release of irritating gas or vapours. Avoid generating dust; fine dust dispersed in air in sufficient concentrations and in the presence of an ignition source is a potential dust explosion hazard. 3. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 4. Potassium sulphate: Hazardous decomposition products are formed under fire like sulphur oxides, potassium oxides. Avoid breathing and formation of dust. Perform all operations in a well ventilated area. 5. Copper sulphate: Non-combustible, substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. 		
Principle	Casein is precipitated from milk at pH 4.6 using acetic acid and sodium acetate solutions in Kjeldahl flask. The acidified solution which contains the non-casein nitrogen components is separated from casein precipitate by filtration. Nitrogen content of the casein precipitate is determined by Kjeldahl method and multiplied by 6.38 to obtain casein in protein.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Kjeldahl apparatus 2. Analytical balance (Readability 0.0001 g) 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Sodium acetate solution. 2. Acetic acid solution. 3. Buffer solution. 		

	<ol style="list-style-type: none"> 4. Sodium acetate trihydrate. 5. Glacial acetic acid. 6. Potassium sulphate. 7. Copper sulphate. 8. Concentrated Sulphuric acid (Approximately 36.8 N).
Preparation of reagents	<ol style="list-style-type: none"> 1. Sodium acetate solution (1 M/ litre) using AR sodium acetate or sodium acetate trihydrate: Transfer 4.10 ± 0.1 g sodium acetate or 6.80 ± 0.1 g sodium acetate trihydrate into 50 mL volumetric flask and dilute to volume with water; prepare fresh weekly. 2. Acetic acid solution (10%) Mix 10 g of acetic acid with 50 mL distilled water and makeup the volume to 100 mL with distilled water. 3. Buffer solution: Dilute 1 ± 0.1 mL sodium acetate and 1 ± 0.1 mL 10.5% acetic acid to 100 mL with water; prepare fresh.
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh 1-2 g of sample into the beaker, add 50 mL water and 0.75 mL of 10% acetic acid to the flask and swirl gently. Leave the mixture for 10 min. 2. Add 0.75 mL of sodium acetate and swirl gently. 3. Filter the mixture on to a pleated filter paper (Whatman No. 1, 15 cm) and collect the filtrate; let it drain completely before the next pour. 4. Add 30 mL of buffer solution to beaker and swirl to mix. 5. Pour the mixture on to the filter paper after the first filtration is complete and combine the filtrates. 6. Add another 30 mL of the buffer solution and add this filtrate to the previous 2 filtrates. 7. The filtrate should be clear and free of particulate matter. 8. If particulates appear, recycle the filtrate through the same filter paper or repeat the test. 9. Remove filter paper; ensure that no precipitate is lost on the filter paper. 10. Drop the filter paper into kjeldahl flask/digestion tube and proceed as per method for determination of protein in milk and milk products.
Calculation with units of expression	Refer the method for determination of protein in milk and milk products (FSSAI 01.026:2021)
Reference	<ol style="list-style-type: none"> 1. AOAC 998.06. 21st Edn. (2019). Official Method Casein Nitrogen Content of Milk). AOAC International, USA. 2. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva.

	<ol style="list-style-type: none">3. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva.4. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Lactose Content in Edible Casein Products (by Photometric Method)		
Method No.	FSSAI 01.116:2021	Revision No. & Date	0.0
Scope	A photometric method for determination of lactose content in edible acid casein, edible rennet casein and edible caseinates is specified		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Phenol: Phenol is highly corrosive to the skin and readily absorbed through it. Work with phenol in a chemical fume hood, especially when heating it. Never heat or melt phenol in an incubator, microwave, drying oven, or similar appliance. Change gloves frequently. Wear chemical goggles to protect the eyes. 		
Principle	The casein is precipitated by addition of acetic acid and sodium acetate solution at pH 4.6; this is followed by filtration which gives a protein-free solution of the carbohydrates. The colour is developed in an aliquot portion of filtrate (by the addition of phenol and concentrated sulphuric acid) which is proportional to the amount of lactose present. The developed colour is read at 490 nm is a spectrophotometer.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Conical flasks: 100 mL capacity. 3. Pipettes: 1, 2 and 10 mL capacities. 4. Graduated pipettes: 25 mL capacity. 5. Volumetric flasks: 100 mL capacity. 6. Test tubes: 40 mL capacity ground neck and fitted with ground glass stoppers. 		

	<ol style="list-style-type: none"> 7. Automatic dispenser: Capable of dispensing 5 mL of concentrated sulphuric acid within 1 sec. 8. Vortex mixer. 9. Micropipettes: 0.2 mL capacity with 0.001 mL divisions. 10. Filter paper: Whatman filter paper Grade 42. 11. Water-baths, maintained at 60 to 70 °C, and at 20 °C. 12. Spectrophotometer, suitable for making measurements at a wavelength 490 nm, provided with cuvettes of optical path length of 1 cm.
Materials and Reagents	<ol style="list-style-type: none"> 1. Sodium hydrogen carbonate (NaHCO₃): For analysis of acid casein. 2. Pentasodium triphosphate (Na₅P₃O₁₀): For analysis of rennet casein. 3. Concentrated hydrochloric acid (Approximately 11.6 N). 4. Acetic acid. 5. Sodium acetate. 6. Phenol. 7. Concentrated sulphuric acid (Approximately 36.8 N). 8. Lactose.
Preparation of reagents	<ol style="list-style-type: none"> 1. Dilute hydrochloric acid (0.1 N): Dilute 8.62 mL of concentrated hydrochloric acid to 1000 mL with distilled water. 2. Dilute sulphuric acid (0.1N): Dilute 2.717 mL of concentrated sulphuric acid to 1000 mL with distilled water. 3. Dilute acetic acid solution (100 g/L): Mix 100 g of acetic acid with 50 mL of distilled water and make up the volume to 100 mL with distilled water. 4. Sodium acetate solution (1 M): Dissolve 82.03 g of sodium acetate in 100 mL of distilled water and make up the volume to 1000 mL with distilled water. 5. Lactose (20 g/L) solution: Weigh 2.105 ± 0.001 g of lactose monohydrate, corresponding to 2.00 g of the anhydrous lactose, into a 100 mL volumetric flask. Dissolve lactose in hot water, make up to volume and mix well. Store the solution at 0 °C. 6. Pheno 80% by mass: Heat a mixture of 8 g of phenol and 2 g of water until the mixture is homogeneous,
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.
Method of analysis	<ol style="list-style-type: none"> 1. Weigh to the nearest 1 mg about 1 g of the test sample into a 100 mL conical flask; add the reagents as the case may be: 2. In the case of acid casein, add 0.1 ± 0.001 g of the sodium hydrogen carbonate. 3. In the case of rennet casein, add 0.1 ± 0.001 g of the pentasodium and 25 mL of water; place the flask in a water-bath controlled at 60 - 70 °C, and mix occasionally by shaking. 4. When the test portion is completely dissolved (after 10 to 15 min), cool

and add successively 15 mL of water, 8 mL of dilute hydrochloric acid or sulphuric acid solution and 1 mL of the dilute acetic acid solution.


5. Stopper and mix the contents by shaking after each addition.
6. Leave for 5 min and then add 1 mL of the sodium acetate solution; mix by shaking.
7. Allow the casein precipitate to settle, and filter through a dry Whatman filter paper Grade 42. Discard the first few mL of the filtrate.
8. Simultaneously, carry out a blank by taking 0.1 ± 0.001 g of sodium hydrogen carbonate or 0.1 ± 0.001 g of pentasodium triphosphate, as appropriate, (the reagent shall be same as used in case of test sample), using the same apparatus and reagents.
9. Pipette 2 mL of the filtrate into a test tube, add 0.2 mL of the phenol solution by means of a micropipette, and mix by shaking.
10. In less than 1 sec, add 5 mL of concentrated sulphuric acid from the automatic dispenser, directing the stream of acid against the liquid surface rather than against the side of the test tube in order to obtain good mixing. Immediately mix, using the mixer, and allow to stand for 15 min. Cool for 5 min in the water bath at 20 °C.
11. Wipe the tube and precede immediately, measure the absorbance of the solution at 490 nm using the blank solution as the reference. If the absorbance is above the upper limit of the calibration graph, repeat using 2 mL of a suitable dilution of the filtrate instead of 2 mL of the filtrate itself.

Note: If such a dilution is made, the formula given for calculation has to be modified accordingly.

Preparation of the Calibration Graph

12. Pipette 10 mL of the lactose solution (20 g/L solution) into a 100 mL volumetric flask and dilute to the mark with water (solution A); 1 mL of solution A corresponds to 2 mg of anhydrous lactose.
13. Prepare three standard solutions by pipetting 1, 2 and 3 mL of solution A into three 100 mL volumetric flasks and making up the volumes with water. The anhydrous lactose concentrations of the standard solution obtained are respectively 20, 40 and 60 µg/mL.
14. Using four test tubes, proceed in accordance, but replace 2 mL of filtrate respectively, by 2 mL of each of the three standard solutions and 2 mL of water.
15. Measure the absorbances of the three standard matching solutions using the solution obtained by treatment of the 2 mL of water as the reference liquid.
16. Construct a calibration curve by plotting the absorbances of the standard

	matching solutions against their anhydrous lactose concentrations in $\mu\text{g/mL}$. Draw the best-fitting line through the calibration points.
Calculation with units of expression	<p>The lactose content of the sample, expressed as anhydrous lactose as a percentage by mass, is calculated using the following formula:</p> $\text{Lactose (\% by mass)} = \frac{C \times 10^{-6} \times 50}{m} \times 100$ <p>Where,</p> <p>c = concentration, in $\mu\text{g/mL}$, of anhydrous lactose in the test solution, read from the calibration curve.</p> <p>m = mass in g, of the test portion.</p>
Reference	IS 11963: 2005 (Reaffirmed year 2019). Method for determination of lactose content by photometric method in caseins and caseinates. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis


 <p>fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Fixed Ash (ash including P₂O₅) Content in Edible Casein Products		
Method No.	FSSAI 01.117:2021	Revision No. & Date	0.0
Scope	This method is used for the determination of fixed ash (ash including P ₂ O ₅) content in casein/caseinates.		
Caution	Follow all safety procedures and wear laboratory apron and safety goggles. <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot crucibles. 2. Hot crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	A test portion of the sample is incinerated at 825 ± 25 °C in the presence of magnesium acetate (added to bind all phosphorus of organic origin). The resulting ash is weighed and mass of the ash originating due to magnesium acetate is subtracted.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Pipette: 5 mL capacity. 3. Silica or platinum dishes about 70 mm diameter and 25 to 50 mm deep. 4. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 5. Electrical furnace with air circulation, capable of being controlled at 825 ± 25 °C. 6. Boiling water-bath. 7. Desiccator, containing an effective desiccant (for example freshly dried silica gel with a hygroscopic indicator). 		
Materials and Reagents	Magnesium acetate tetra hydrate [Mg (CH ₃ CO ₂) ₂ .4H ₂ O].		
Preparation of reagents	Magnesium acetate tetra hydrate (120 g/L solution): Weigh 120 g of magnesium acetate and dissolved in 1L of water.		
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation		
Method of analysis	<ol style="list-style-type: none"> 1. Heat two dishes in an electrical furnace controlled at 825 ± 2 °C for 30 min. 2. Allow the dishes to cool in the desiccator to the 25 ± 3 °C and weigh to the nearest 0.1 mg. 3. Weigh to the nearest accuracy (0.1 mg directly in or by difference) approximately 3 g of the test sample into one of the prepared dishes (A). 4. Using a pipette, add to the dish (A) exactly 5 mL of the magnesium acetate solution so as to wet the entire portion and allow it to stand for 20 min. To the other prepared dish (B), add 5 mL of magnesium acetate 		

	<p>solution.</p> <ol style="list-style-type: none"> 5. Evaporate the contents of both dishes (A and B) to dryness on the boiling water bath. 6. Place both the dishes in the oven controlled at 102 ± 2 °C for 30 min. 7. Heat dish A with its content on a low flame until the test portion is completely charred, taking care that it does not burst into flame. 8. Transfer both the dishes (A and B) to the electrical furnace controlled at 825 ± 25 °C, and heat for at least 1 h until all carbon has disappeared from dish A. Allow both the dishes to cool in the desiccator to the room temperature (25 ± 3 °C) and weigh to the nearest 0.1 mg. 9. Repeat the operations of heating in the electrical furnace, cooling and weighing, until the mass remains constant to within 1 mg. Record the minimum mass. <p>Determination of moisture</p> <p>Refer method for determination of moisture in edible casein products (FSSAI 01.112:2021).</p>
<p>Calculation with units of expression</p>	<p>The “fixed ash” of the sample, including phosphorus, as a percentage by mass is calculated by the following formula:</p> $\text{Fixed Ash, } W_{fa} = \frac{(M_1 - M_2) - (M_3 - M_4)}{M_0} \times 100$ <p>Where,</p> <p>M_0 = mass in g of the test portion; M_1 = mass in g of dish A and residue; M_2 = mass in g of the prepared empty dish A; M_3 = mass in g of the dish B and residue; M_4 = mass in g of the prepared empty dish B.</p> <p>Calculate the “fixed ash” to the nearest 0.01% and report the final result to the nearest 0.1%.</p> <p>To calculate the “fixed ash” of the sample on the dry basis, as a percentage by mass, multiply the result obtained as above by</p> $= \frac{100}{100 - M}$ <p>where,</p> <p>M is the % moisture content of the sample.</p>
<p>Reference</p>	<p>IS 11919: 1987 (Reaffirmed year 2019). Method for determination of fixed ash in rennet caseins and Caseinate (Reference Method). Bureau of Indian Standards, New Delhi)</p>
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>


Determination of Free Acidity in Edible Casein Products


Method No.	FSSAI 01.118:2021	Revision No. & Date	0.0
Scope	This method is for the determination of free acidity in casein/caseinates.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 2. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 		
Principle	Free acidity in casein is the volume in mL of 0.1 N standard sodium hydroxide solution required to titrate an aqueous extract of 1 g of casein sample using phenolphthalein as indicator. This is determined by aqueous extraction of test portion of the sample at 60 °C followed by filtration and then titration of the filtrate with a standard sodium hydroxide solution to the end point of phenolphthalein.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.001 g). 2. Conical flask: 500 mL capacity, with ground neck and fitted with a ground glass stopper and 200 mL capacity. 3. Burette; 50 mL capacity, graduated in 0.1 mL. 4. Measuring cylinder: 100 mL, 250 mL capacity. 5. Water-bath maintained at a temperature of 60 ± 2 °C. 6. Appropriate filter. 		
Materials and Reagents	<ol style="list-style-type: none"> 1. Sodium hydroxide. 2. Phenolphthalein indicator. 3. Potassium hydrogen phthalate. 4. Ethanol. 		
Preparation of reagents	<ol style="list-style-type: none"> 5. Standard sodium hydroxide solution: 0.1 N standardised against primary standard potassium hydrogen phthalate. 6. Phenolphthalein indicator: Dissolve 1 g of phenolphthalein in 100 mL of 95% ethanol (v/v). 		
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh about 10 g of the test sample to the nearest 10 mg and transfer it to a conical flask (500 mL capacity). 		

	<ol style="list-style-type: none"> 2. Add 200 mL of water (at 60 °C and already boiled). Stopper the flask, mix it by swirling and place in the water-bath at 60 °C for 30 min. 3. Shake the flask at intervals of about 10 min. 4. Filter the contents and cool the filtrate to about 20 °C. The filtrate must be clear. 5. Transfer 100 mL of the cool filtrate into the conical flask (200 mL capacity) using a measuring cylinder. 6. Add 0.5 mL of the ethanolic phenolphthalein solution using a pipette. 7. Titrate with standard Sodium hydroxide solution until a faint pink colour appears; the colour should persist for at least 30 sec. 8. Record the volume used to 0.01 mL.
Calculation with units of expression	<p>The free acid of the casein is calculated by using the following formula: Free acidity (mL of 0.1 N sodium hydroxide per g sample) = $\frac{20 \times V \times T}{M}$</p> <p>Where, V = volume, in mL, of the standard sodium hydroxide solution used; T = normality of the standard sodium hydroxide solution; M = mass in g, of the test portion. Calculate the free acid to the nearest 0.01 mL.</p>
Reference	IS 11964: 2012 (Reaffirmed year 2018). Method for determination of free acidity in rennet caseins (Reference Method). Bureau of Indian Standards, New Delhi).
Approved by	Scientific Panel on Methods of Sampling and Analysis


	Determination of pH in Edible Casein Products		
Method No.	FSSAI 01.119:2021	Revision No. & Date	0.0
Scope	This method describes the determination of pH in casein/caseinates.		
Caution	Wear laboratory apron, shoes, safety goggles and mask while working in laboratory.		
Principle	In this method, pH of an aqueous extract of casein or an aqueous solution of caseinate is determined at 20 °C, using a calibrated pH meter.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. pH meter, minimum sensitivity 0.05 pH unit, with a suitable glass electrode and a calomel or other reference electrode. 2. Thermometer, with an accuracy of ± 0.5 °C. 3. Conical flask: 100 mL capacity, fitted with a ground glass stopper. 4. Beaker: 50 mL capacity. 5. Mixer. 6. Beaker (for the mixer), of at least 250 mL capacity. 		
Materials and Reagents	Buffer solutions, for calibration of the pH meter: Two standard buffer solutions with pH values at 20 °C which are known to the second decimal place and will bracket the pH value of the sample under test, for example phthalate buffer solution of pH approximately 4 and a borax buffer solution of pH approximately 9. In addition, a phosphate buffer solution of pH approximately 7 may be used.		
Sample preparation	Refer method FSSAI 01.111:2021 for sample preparation.		
Method of analysis	<p>Calibration of pH meter: Adjust the temperature of the buffer solutions to 20 °C and calibrate the pH meter in accordance with the manufacturer's instructions.</p> <p>Notes If a series of samples is being tested, check the calibration of the pH meter with one or more of the standard buffer solutions at least every 30 min.</p> <p>Preparation of test solution</p> <ol style="list-style-type: none"> 1. Prepare 10% solution of sample by dissolving 10 g of sample in distilled water and making the final volume to 100 mL. 2. Determine pH with calibrated pH meter. <p>Recording of pH Caseins: Record (as the pH of the aqueous extract of casein) the value read to at least one decimal place. Caseinates: Record (as the pH of the aqueous solution of caseinate) the value read to at least two decimal places.</p>		
Reference	IS 11918: 2017. Method for determination of pH in caseins and caseinates (Reference Method). Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

O. COLOSTRUM

	Preparation of Sample of Colostrum		
Method No.	FSSAI 01.120:2021	Revision No. & Date	0.0
Scope	This method describes the preparation of a colostrum sample for analysis		
Sample preparation	<ol style="list-style-type: none"> 1. Warm the sample to 37 - 40 °C by transferring it to the beaker and keeping it in a water bath maintained at 40 - 45 °C. 2. Stir slowly for proper homogenisation, mix sample thoroughly by pouring back into the bottle, mixing to dislodge any residual fat sticking to the sides and pour it back in the beaker. 3. During mixing do not shake the bottle vigorously. 4. Allow the sample to come to room temperature (26 - 28 °C) and withdraw immediately for analysis. 		
Reference	IS 1479 (Part II):1961 (Reaffirmed year 2018). Methods of test for Dairy Industry -Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

		Determination of Moisture content in Colostrum	
Method No.	FSSAI 01.121:2021	Revision No. & Date	0.0
Scope	This method describes the determination of moisture content of colostrum.		
Caution	<p>Follow all safety procedures and wear laboratory apron and safety goggles.</p> <ol style="list-style-type: none"> 1. Use thermal protection gloves, tongs and protective eyewear while handling hot moisture dishes. 2. Hot moisture dishes will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 		
Principle	In this method, first total solids are determined in the sample. For estimating moisture content total solids are subtracted from 100.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Analytical balance (Readability 0.0001 g). 2. Flat-bottomed dishes: Made of aluminum alloy, nickel, stainless steel, porcelain, 50 to 75 mm diameter, 20 to 25 mm depth, and provided with easily removable but closely fitting lids. 3. Boiling water-bath (100 ± 2 °C). 4. Hot air oven (forced air type), capable of being controlled at 102 ± 2 °C. 5. Desiccator with efficient desiccant (for example freshly dried silica gel with a hygroscopic indicator). 6. Short glass stirring rods: Flattened at one end and fitting in to the dish. 		
Sample preparation	Refer method FSSAI 01.120:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Take a dish with the lid and stirring rod and weigh to the nearest 0.1 mg. 2. Place 2.0 g of the prepared test sample in the dish, spread evenly with the help of stirring rod and weigh the dish to the nearest 0.1 mg. 3. Heat the dish on a boiling water-bath, for approximately 30 min while stirring 4. Dry the bottom of the dish and heat the dish with its lid alongside, in a hot air oven maintained at 102 ± 2 °C for 4 h. 5. Place the lid on the dish, allow the dish to cool in the desiccator and weigh to the nearest 0.1 mg. Repeat the operations described above (heating the dish for 1 h) until the difference in mass between two successive weighing does not exceed 0.5 mg. 6. Record the lowest mass. 		
Calculation with units of expression	<p style="text-align: center;">Moisture % by mass = $\frac{M_1 - M_2}{M_1 - M} \times 100$</p> <p>Where,</p>		

	<p>M is mass in g, of the empty dish with lid along with glass rod; M₁ is initial mass in g of the dish with lid, glass rod, and test portion; M₂ is the mass in g of the dish with lid, glass rod, and dried test portion Express the results to the nearest 0.01% (m/m).</p>
Reference	IS 1479 (Part II):1961 (Reaffirmed year 2018). Methods of test for Dairy Industry -Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Fat Content in Colostrum (Gravimetric method)		
Method No.	FSSAI 01.122:2021	Revision No. & Date	0.0
Scope	This method describes the determination of fat content in colostrum.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Ammonia solution: Do not breathe vapor or mist. Use only with adequate ventilation. Although ammonia is not flammable, it may ignite and burn with explosive force. It reacts violently with halogens (such as fluorine, bromine, and chlorine), acids (such as hydrochloric acid, hydrogen fluoride, hydrogen bromide). 2. Ethyl alcohol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 3. Diethyl ether: Store protected from light. It is extremely flammable and can react explosively when in contact with Cl₂, O₃, LiAlH₄ or strong oxidizing agents. 4. Petroleum ether: Extremely flammable. Avoid breathing fumes. Perform all operations in a fume hood. 		
Principle	Rose-Gottlieb Method is a gravimetric method in which fat globule membrane of milk fat globules is ruptured by addition of ammonia to milk sample to liberate the fat. The liberated fat is extracted using combination of solvents viz., diethyl ether and petroleum ether. The solvents are evaporated and obtained fat is dried and weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Mojonnier fat extraction flask or any other suitable extraction tube (as per IS specification). 2. Cork or stopper of synthetic rubber unaffected by usual fat solvents. 3. 100 mL flat bottom flask with G/G joint or stainless steel or aluminium dishes of 5.5 cm height and 9 cm diameter or glass bowl. 		
Materials and reagents	<ol style="list-style-type: none"> 1. Ammonia solution (Specific gravity - 0.91). 2. Ethyl alcohol (95%). 3. Diethyl ether, peroxide-free. 4. Petroleum ether, boiling range 40-60 °C. 		
Sample preparation	Refer method FSSAI 01.120:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh accurately about 10 g of sample in a beaker. 		

	<ol style="list-style-type: none"> 2. Add 1.25 mL of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used), mix and shake thoroughly. 3. Transfer the content to extraction tubes. 4. Proceed for the extraction of fat by Rose-Gottlieb method as prescribed for milk from the step indicating addition of 10 mL ethyl alcohol (Method No FSSAI 01.23:2021).
Calculation with units of expression	$Fat \% (m/m) = \frac{(M_1 - M_2) - (M_3 - M_4)}{w} \times 100$ <p>Where,</p> <p>M₁ is the mass, in g, of the fat-collecting flask and extracted matter after drying M₂ is the mass, in g, of the empty fat-collecting flask M₃ is the mass, in g, of the fat-collecting flask used in the blank test after drying M₄ is the mass, in g, of the empty fat-collecting flask used in the blank test w is the weight of the sample in g</p>
Reference	<ol style="list-style-type: none"> 1. F.A.O. Manual of Food Quality Control, 14/8, page 8. 2. IS 1479 (Part II): 1961 (Reaffirmed Year: 2018). Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi. 3. AOAC 905.02. 21st Edn. (2019). Official method –Fat in milk. AOAC International, USA. 4. ISO 1211: 2010. Milk — Determination of fat content — Gravimetric method (Reference method). International Organization for Standardization, Geneva. 5. Pearson’s Composition and analysis of foods, 9th edn., 1991 page 538.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Determination of Milk Protein in Colostrum

Method No.	FSSAI 01.123:2021	Revision No. & Date	0.0
Scope	This method describes the determination of milk protein in colostrum.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa. 2. Concentrated sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. It reacts violently with water with evolution of heat; can react with organic materials explosively. Do not mix with hydrochloric acid. Good general ventilation should be provided to keep vapour and mist concentrations below the exposure limits. 3. Ethanol: Extremely flammable. Use effective fume removal device when heating or evaporating. Keep away from heat, sparks and open flame; avoid breathing vapours. 4. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 5. Ammonium sulphate: Thermal decomposition products can lead to release of irritating gases and vapours. Minimize dust generation and accumulation. 		
Principle	Refer the method for determination of protein by Kjeldahl method in milk and milk products (FSSAI 01.025/026:2021)		
Apparatus/Instruments			
Materials and reagents			
Preparation of Reagents			
Sample preparation	Refer method FSSAI 01.120:2021 for sample preparation.		
Method of analysis	<ol style="list-style-type: none"> 1. Weigh quickly about 3-4 g of the prepared colostrum sample and transfer to a 500 or 800 mL Kjeldahl flask/digestion tube taking care to see that no portion of the sample clings to the neck of the flask/tube. 2. Refer the method for determination of protein in milk and milk products 		

	(FSSAI 01.025/026:2021).
Calculation with units of expression	Refer the method for determination of protein in milk and milk products (FSSAI 01.025/026:2021)
Reference	<ol style="list-style-type: none"> 1. ISO 8968-1/IDF 020-1:2001. Milk - Determination of nitrogen content (Part 1): Kjeldahl method. International Organization for Standardization, Geneva. 2. ISO 8968-2/IDF 020-2:2001. Milk - Determination of nitrogen content (Part 2): Block-digestion method (Macro method). International Organization for Standardization, Geneva. 3. IS 7219: 1973 (Reaffirmed year 2015). Method for determination of protein in foods and feeds. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	Determination of Melamine and Cyanuric acid in Milk, Milk Products and Infant formula		
Method No.	FSSAI 01.124:2021	Revision No. & Date	0.0
Scope	This method describes the determination of melamine in milk and milk products and is applicable for infant formula.		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Melamine: Keep away from heat. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, acids. 2. Acetonitrile: Do not inhale substance/mixture. Avoid generation of vapours/aerosols. Keep away from open flames, hot surfaces and sources of ignition. Keep container tightly closed in a dry and well-ventilated place (+5 °C to +30 °C). 3. Formic acid: Do not inhale substance/mixture. Keep away from open flames, hot surfaces and sources of ignition. May decompose forming gaseous products, especially when stored over long periods, so place in close containers in such a way to enable internal pressure to escape (e.g. excess pressure valve). Protected from light. Keep container tightly closed in a dry and well-ventilated place. 4. Ammonium formate: Ensure adequate ventilation. Avoid dust formation. Keep containers tightly closed in a dry, cool and well-ventilated place. Incompatible with strong oxidizing agents, strong acids and strong bases. 		
Principle	A liquid chromatography triple quadrupole tandem mass spectrometry (LC-MS/MS) method for residues of melamine consists of an initial extraction with 2.5% aqueous formic acid, followed by a series of filtration, centrifugation, and dilution steps. The method detects both melamine and cyanuric acid using a Hydrophilic Interaction Liquid Chromatography (<i>HILIC</i>) column and mass spectroscopy. Melamine is detected in positive ion mode and cyanuric acid in negative ion mode. Analyte concentrations are calculated using an external standard calibration curve prepared using pre-fortified control matrix, which has been carried through the extraction procedure.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. Liquid chromatograph: Binary LC pump is recommended for accurate mixing at low flow rate and rapid response to mobile phase gradient. 2. Column: ZIC-HILIC, 2.1 × 150 mm, 5 µm, 200 Å or equivalent. 3. Mass Spectrometer: Triple quadrupole capable of meeting system suitability. 4. Analytical balance (Readability 0.0001 g). 5. Centrifuge: Capable of 4000 rpm (approximately 3750 g) with 50 mL 		

	<p>tubes.</p> <ol style="list-style-type: none"> 6. Microcentrifuge: Capable of 13,000 rpm (16000 g) with 1.5 /2 mL tubes. 7. Mixers and shakers: Single and multi-tube vortex mixers platform shaker 8. Ultrasonic bath: Including timer and heater. 9. Centrifuge tubes: 50 mL disposable polypropylene with caps, with graduations from 5 to 50 mL and 1.5 mL microcentrifuge tubes. 10. Syringe Filters: Polyvinylidene fluoride (PVDF), 13 mm, 0.22 µm. 11. Syringes: Three mL polypropylene.
Materials and Reagents	<ol style="list-style-type: none"> 1. Melamine (CAS #: 108-78-1). 2. Acetonitrile (LC grade). 3. Formic acid (Reagent grade > 95%). 4. Water (LC grade, or purified by Millipore Milli-Q system to > 18 Mohm resistivity, or equivalent). 5. Ammonium formate (Purity > 97%).
Preparation of reagents	<ol style="list-style-type: none"> 1. 0.1% Formic acid in water: One mL formic acid is transferred to one L graduated flask and diluted to volume with LC water. 2. Mobile phase A: 0.1% Formic acid in acetonitrile (5:95 v/v). Mix 50 mL of 0.1% formic acid in water with 950 mL acetonitrile in a one L solvent bottle. 3. Mobile phase B: 20 mM Ammonium formate in acetonitrile (50:50 v/v). Mix 500 mL of 20 mM ammonium formate and 500 mL of acetonitrile in a one L solvent bottle. 4. 2.5% Formic acid in water: 25 mL formic acid is transferred to one L volumetric flask and diluted to volume with LC grade water. 5. 20 mM Ammonium formate: 0.63 gm of ammonium formate is weighed and dissolved in 500 mL LC grade water.
Sample preparation	Refer appropriate method for sample preparation for the particular product.
Method of analysis	<ol style="list-style-type: none"> 1. Sample of milk/milk products (2.0 ± 0.1 g) is weighed in a 50 mL polypropylene centrifuge tube. 2. Pre-fortify control and matrix calibration standards 3. 14 mL of 2.5% Formic acid in water is added to samples. Tube is tightly sealed. Dissolve sample by shaking for 15-30 seconds (vortex as needed), then sonicate in ultrasonic bath and mix on multi vortex mixer for 30 min each. 4. Centrifuge at 4000 rpm (3750 g) for 10 min at 25 ± 3 °C. 5. Approximately 1.4 mL of the supernatant is transferred into a 1.5 mL micro centrifuge tube. 6. Centrifuge at 13,200 rpm (16100 g) for 30 min. 7. Load aqueous extract into a plastic 3 mL syringe and force through a 13 mm, 0.22 µm PVDF filter into a micro centrifuge tube. (Note: some

	<p>formulations may require some force, or two filtration steps to obtain a clear solution before the next step.). Possible stopping point: aqueous extracts can be stored at 5 - 10 °C for future dilutions.</p> <p>8. Vortex mix for 30 seconds and centrifuge at 13200 rpm (16100 g) for 30 min.</p> <p>9. Supernatant is transferred to a 2 mL autosampler vial, avoiding the precipitate.</p> <p>Instrumental Analysis</p> <p>The column is equilibrated in mobile phase A at 0.4 mL/min for 30 - 60 min. It is necessary to evaluate system suitability, solvent blank (1x) and mixed standard are injected at 7.0 ng/mL (3-4x).</p> <p>Data should meet the signal-to-noise and ion ratio criteria before continuing. It is recommended to inject the standards and sample in following sequence: (i) solvent blank (mobile phase A), (ii) extracted matrix standards from 0.25 to 5 µg/g, (iii) solvent blank, (iv) control extracts, (v) post-fortified extracts and solvent standards for calculation of recoveries and matrix effects, (vi) solvent blank, (vii) unknown samples, and (viii) continuous calibration standards (an extracted matrix standard as well as solvent standard at 7 ng/mL), to verify that instrument response was maintained during the run.</p>
<p>Calculation with units of expression</p>	<p>Use external standard calibration. The calibration curve should not include the origin, but does include a matrix blank with a concentration of 0. Export the processed data into Microsoft Excel or equivalent spreadsheet program for further calculations:</p> <p>Recovery (%) = calculated from extracted calibration curve</p> <p>Matrix effect (%) = 100 x Post-fortified sample / solvent standard (same concentration)</p> <p>The limit of quantification (LOQ) for each analyte is defined as the concentration of the lowest calibration standard used, or the lowest calibration standard which shows > 10-fold higher response than background signals in negative control sample.</p> <p>Calculations for Confirmatory Analysis</p> <p>Calculate ion ratios as percent relative abundances. The melamine ion ratio is m/z 68/85.</p>
<p>Reference</p>	<p>Turnipseed, S., Casey, C., Nochetto, C., & Heller, D. N. (2008). Determination of melamine and cyanuric acid residues in infant formula using LC-MS/MS. US FDA laboratory information bulletin, 24, 4421.</p>
<p>Approved by</p>	<p>Scientific Panel on Methods of Sampling and Analysis</p>

ANNEXURES

 Preparation of Standard Solution of Sodium Hydroxide	
Method No.	FSSAI 01.125:2021 Revision No. & Date 0.0
Scope	This scope is for the preparation of standard solution of sodium hydroxide
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> Sodium hydroxide: It is extremely caustic and can cause severe burns. Protect skin and eyes when working with these alkalis as solids or concentrated solutions. While making solution add pellets to water and not vice versa.
Principle	<p>Since sodium hydroxide is not a standard substance, so to prepare a standard solution of sodium hydroxide, it is required to be standardized against the primary standard, oxalic acid/potassium acid phthalate to accurately determine its normality. When an acid reacts with an alkali, salt and water are formed and the reaction is known as neutralization. An indicator indicates the end point by its characteristic colour change, such as colorless to pink when phenolphthalein is used as indicator and change in color occurs at pH 8.3</p> $2 \text{NaOH} + \text{H}_2\text{C}_2\text{O}_4 \longrightarrow \text{Na}_2\text{C}_2\text{O}_4 + 2\text{H}_2\text{O}$ <p>(Sodium hydroxide) (Oxalic acid) (Sodium Oxalate)</p>
Apparatus/ Instruments	<ol style="list-style-type: none"> Beaker (100 mL). Volumetric flask (100 mL). Burette (25 mL). Analytical balance (Readability 0.0001 g).
Materials and reagents	<ol style="list-style-type: none"> Sodium hydroxide. Oxalic acid. Potassium acid phthalate. Phenolphthalein indicator.
Preparation of reagents	<p>A) Preparation of 100 mL of 0.1 N sodium hydroxide solution</p> <ol style="list-style-type: none"> Weigh approximately 0.42 g of NaOH pellet and dissolve it in 50 mL of distilled water in a clean dry beaker. Then transfer the above 50 mL of solution into a 100 mL volumetric flask. Make the volume upto 100 mL using distilled water. The solution is approximately 0.1 N (equivalent weight of NaOH=40.01)

	<p>1. Preparation of 100 mL of 0.1 N oxalic acid solution</p> <ol style="list-style-type: none"> 1. Weigh 0.63 g of oxalic acid (molecular weight=126.07) and dissolve in 70-80 mL of distilled water. 2. Thereafter make the volume to 100 mL using distilled water. This solution is 0.1N oxalic acid. <p>2. Preparation of 0.1 N Potassium Acid Phthalate</p> <ol style="list-style-type: none"> 1. 2.042 g of potassium acid phthalate (Molecular weight 204.2) is weighed in 100 mL volumetric flask and dissolve in 70-80 mL of distilled water. 2. Thereafter make the volume to 100 mL using distilled water. This solution is 0.1N potassium acid phthalate. <p>3. Phenolphthalein indicator (1%): Dissolve 1 g phenolphthalein in 100 mL 95% ethanol.</p>
<p>Method of analysis</p>	<ol style="list-style-type: none"> 1. Fill the burette with prepared 0.1 N sodium hydroxide upto a known mark. 2. Pipette out 10 mL of prepared 0.1N oxalic acid/ potassium acid phthalate solution in a beaker. 3. To it add 2-3 drops of phenolphthalein indicator 4. 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. 5. Note down the final reading on the burette, repeat the titration till at least three concordant readings are obtained. 6. Determine the actual normality of the prepared sodium hydroxide solution using the volume of sodium hydroxide solution consumed to neutralize 10 mL of oxalic acid/potassium acid phthalate using following equation: <p style="text-align: center;">$N_1V_1 = N_2V_2$</p> <p>Where</p> <p>N_1 = normality of standard oxalic acid /potassium acid phthalate (0.1)</p> <p>V_1 = volume in mL of oxalic acid /potassium acid phthalate used for titration (10 mL)</p> <p>N_2 = normality of prepared sodium hydroxide (to be determined)</p> <p>V_2 = volume in mL of prepared sodium hydroxide used in the titration</p> 7. If the Normality of the sodium hydroxide calculated is not same as intended, then dilute it accordingly to have required normality (0.1 N sodium hydroxide Solution) using the following equation <p style="text-align: center;">$N_aV_a = N_bV_b$</p> <p>Where,</p> <p>N_a = required normality of the solution</p>

	<p> V_a = required volume of solution N_b = Normality of prepared solution V_b = Volume of prepared solution to be dilute </p> <p>Volume of N_b N sodium hydroxide required to dilute it to have 0.1N sodium hydroxide solution =</p> $\frac{100 \times 0.1}{N_b} = \text{'X' ml of given solution}$ <p>8. To check the normality of the final sodium hydroxide solution, again titrate it against 0.1 N oxalic acid and if the volume of oxalic acid/potassium acid phthalate consumed is equal to the volume of sodium hydroxide taken, then the solution is standardized one.</p> <p>9. For preparation of other concentration of sodium hydroxide, the solution can be prepared by weighing appropriate quantity (weight required in g= Equivalent weight x Normality to be prepared x Volume to be prepared in lit) and can be standardized using equivalent concentration of oxalic acid/potassium acid phthalate.</p>
Reference	<ol style="list-style-type: none"> 1. AOAC International. 1995. Official Methods of Analysis of AOAC International, 16th ed. Method 936.16, App. A, p. 6. The Association, Arlington, VA. 2. IS 2316: 1990 (Reaffirmed year 2001). Methods of preparation of standard solutions for colorimetric and volumetric analysis. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Preparation of Standard Solution of Hydrochloric Acid

Method No.	FSSAI 01.126:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of standard solution of hydrochloric acid		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Concentrated hydrochloric acid: Take precautions like wearing a chemical-resistant apron, chemical-resistant gloves and chemical splash goggles at all times when handling Hydrochloric acid to protect your eyes and skin. Concentrated hydrochloric acid is toxic if inhaled, so avoid breathing it in and always handle it while under a fume hood. It reacts with strong oxidizing agents to form toxic chlorine gas. 2. Sodium Carbonate: If improperly handled, or in the event of accidental exposure, sodium carbonate can pose several hazards to people's health and safety. Inhalation of this chemical can lead to adverse effects such as respiratory tract irritation, coughing, shortness of breath, and pulmonary edema. 3. Safety glasses are to be worn whenever working with acids, bases, or any flammable materials. 4. Let the instructor know immediately about any glassware breakage or chemical spills that may occur so that proper cleanup procedures can be instituted. 5. Use pipet fillers whenever pipetting any fluid. Whenever working with acids or bases, have the chemicals available at your work station, do not carry pipets with fluid across the room. 6. When diluting acids, pour the acid into the water, NOT water into acid as this may cause spattering of the acid. 7. When acid is mixed with another reagent, an exothermic reaction may occur which heats up the container, this is natural, so do not panic and spill contents. 		
Principle	<p>The solution of hydrochloric acid is required to be standardized against the primary standard, sodium carbonate to accurately determine its concentration. When an acid reacts with an alkali, salt, water is formed and carbon dioxide is released. When a solution of a weak alkali is titrated against a strong acid, methyl orange indicator should be used.</p> $\text{Na}_2\text{CO}_3 + 2\text{HCl} \longrightarrow 2\text{NaCl} + \text{H}_2\text{O} + \text{CO}_2$ <p style="text-align: center;">(Sodium carbonate) (Hydrochloric acid) (Sodium chloride)</p>		

Apparatus/ Instruments	<ol style="list-style-type: none"> 1. Beaker (100 mL). 2. Volumetric flask (100 mL). 3. Burette (25 mL). 4. Analytical balance (Readability 0.0001 g).
Materials and reagents	<ol style="list-style-type: none"> 1. Hydrochloric acid. 2. Sodium carbonate. 3. Methyl orange indicator.
Preparation of reagents	<p>Preparation of 100 mL of 0.1 N hydrochloric acid solution</p> <ol style="list-style-type: none"> 1. Calculate the normality (N_1) of the given stock solution of the hydrochloric acid using specific gravity and assay as given on the label of the bottle by using the formula: 2. $Normality\ of\ the\ stock\ acid\ solution\ (N_1) = \frac{Specific\ Gravity}{Equivalent\ weight} \times 1000 \times \% \text{ assay}$ 3. Calculate the volume (V_1) of N_1 N hydrochloric acid solution required to dilute it to have 100 mL of 0.1 N hydrochloric acid solutions. 4. Take V_1 mL of the stock solution and make the volume upto 100 mL using distilled water to have 100 mL of 0.1 N hydrochloric acid solution. <p>Preparation of 100 mL of 0.1 N sodium carbonate solution</p> <ol style="list-style-type: none"> 1. Weigh 0.53 g of sodium carbonate (molecular weight =106) and dissolve it in 70-80 mL of distilled water. 2. Thereafter make the volume to 100 mL using distilled water. This solution is 0.1N sodium carbonate. <p>Methyl orange indicator (0.4 g/L). Dissolve 0.1 g of methyl orange in 50 mL of rectified spirit and dilute to 250 mL with distilled water.</p>
Method of analysis	<ol style="list-style-type: none"> 1. Fill the burette with prepared 0.1 N hydrochloric acid upto a known mark. 2. Pipette out 10 mL of prepared 0.1N sodium carbonate solution in a beaker. 3. To it add 2-3 drops of methyl orange indicator. 4. Note down the initial reading on the scale of burette and then titrate with hydrochloric acid solution until the colour changes into a permanent orange (pink) colour from pale yellow. 5. Note down the final reading on the burette, repeat the titration till at least three concordant readings are obtained. 6. Determine the actual normality of the prepared hydrochloric acid solution using the volume of hydrochloric acid solution consumed to neutralize 10 mL of sodium carbonate using following equation: $N_1V_1 = N_2V_2$ <p>Where</p>

	<p> N_1 = normality of standard sodium carbonate (0.1) V_1 = volume in mL of sodium carbonate used for titration (10 mL) N_2 = normality of prepared hydrochloric acid (to be determined) V_2 = volume in mL of prepared hydrochloric acid used in the titration </p> <p>7. If the Normality of the hydrochloric acid calculated is not same as intended, then dilute it accordingly to have required normality (0.1 N hydrochloric acid solution) using the following equation</p> $N_a V_a = N_b V_b$ <p>Where,</p> <p> N_a = required normality of the solution V_a = required volume of solution N_b = Normality of prepared solution V_b = Volume of prepared solution to be dilute </p> <p>Volume of N_b N hydrochloric acid required to dilute it to have 0.1N hydrochloric acid solution =</p> $\frac{100 \times 0.1}{N_b} = 'X' \text{ ml of given solution}$ <p>8. To check the normality of the final hydrochloric acid solution, again titrate it against 0.1 N sodium carbonate and if the volume of hydrochloric acid consumed is equal to the volume of sodium carbonate taken, then the solution is standardized one.</p> <p>9. For preparation of other concentration of hydrochloric acid, the solution can be prepared by diluting appropriate quantity ($V = \text{Normality of required hydrochloric acid} \times \text{Volume to be prepared in mL} / \text{Normality of concentrated hydrochloric acid}$) and can be standardized using equivalent concentration of sodium carbonate.</p>
Reference	<ol style="list-style-type: none"> 1. AOAC International. 1995. Official Methods of Analysis of AOAC International, 16th ed. Method 936.15; Appendix A, p. 3. The Association, Arlington, VA. 2. IS 2316: 1990 (Reaffirmed year 2001). Methods of preparation of standard solutions for colorimetric and volumetric analysis. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis

Preparation of Standard Solution of Silver Nitrate

Method No.	FSSAI 01.127:2021	Revision No. & Date	0.0
Scope	This scope is for the preparation of standard solution of silver nitrate		
Caution	<p>Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Refer to MSDS (Material Safety Data Sheets) for specific information.</p> <ol style="list-style-type: none"> 1. Silver nitrate: It is corrosive. It is not combustible, but it is a strong oxidizer that enhances the combustion of other substances. 2. Potassium chromate: It is a strong oxidizing agent. It is a carcinogen and mutagen and should be handled with extreme caution. 		
Principle	<p>The solution of silver nitrate is required to be standardized against the primary standard, sodium chloride and indicator potassium chromate to accurately determine its concentration. Silver nitrate reacts with sodium chloride and forms a white precipitate of silver chloride. When all the chloride ions are precipitated as silver chloride, excess silver nitrate reacts with potassium chromate indicator and forms a reddish-brown precipitate of silver chromate.</p> $2\text{Ag}^+ + \text{CrO}_4^{2-} \longrightarrow \text{Ag}_2\text{CrO}_4(\text{s})$		
Apparatus/ Instruments	<ol style="list-style-type: none"> 1. Beaker (100 mL). 2. Volumetric flask (100 mL). 3. Burette (25 mL). 4. Analytical balance (Readability 0.0001 g). 		
Materials and reagents	<ol style="list-style-type: none"> 1. Sodium chloride. 2. Silver nitrate. 3. Potassium chromate indicator. 		
Preparation of reagents	<p>A) Preparation of 100 mL of 0.1 N silver nitrate solution</p> <ol style="list-style-type: none"> 1. Weigh approximately 1.7 g of silver nitrate (molecular weight = 169.87) and dissolve it in 50 mL of distilled water in a clean dry beaker. 2. Then transfer the above 50 mL of solution into a 100 mL volumetric flask. 3. Make the volume upto 100 mL using distilled water. The solution is approximately 0.1 N. <p>B) Preparation of 100 mL of 0.1 N sodium chloride solution</p> <ol style="list-style-type: none"> 1. Weigh 0.58484 g of sodium chloride (molecular weight = 58.44) and dissolve in 70 - 80 mL of distilled water. 		

	<p>2. Thereafter make the volume to 100 mL using distilled water. This solution is 0.1N sodium chloride.</p> <p>C) 5% Potassium Chromate Solution: Weigh 5 g of potassium chromate in a beaker and dissolve it in distilled water. Transfer the contents of beaker to a 100 mL volumetric flask and make up the volume up to the mark.</p>
<p>Method of analysis</p>	<ol style="list-style-type: none"> 1. Fill the burette with prepared 0.1 N silver nitrate upto a known mark. 2. Pipette out 10 mL of prepared 0.1 N sodium chloride solution in a beaker. 3. To it add 2-3 drops of potassium chromate indicator 4. Note down the initial reading on the scale of burette and then titrate with silver nitrate solution until the red colour precipitates appears. 5. Note down the final reading on the burette, repeat the titration till at least three concordant readings are obtained. 6. Determine the actual normality of the prepared silver nitrate solution using the volume of silver nitrate solution consumed to neutralize 10 mL of sodium chloride using following equation: $N_1V_1 = N_2V_2$ <p>Where</p> <p>N_1 = normality of standard sodium chloride (0.1) V_1 = volume in mL of sodium chloride used for titration (10 mL) N_2 = normality of prepared silver nitrate (to be determined) V_2 = volume in mL of prepared silver nitrate used in the titration</p> 7. If the Normality of the silver nitrate calculated is not same as intended, then dilute it accordingly to have required normality (0.1 N silver nitrate) using the following equation $N_aV_a = N_bV_b$ <p>Where,</p> <p>N_a = required normality of the solution V_a = required volume of solution N_b = Normality of prepared solution V_b = Volume of prepared solution to be dilute</p> <p>Volume of N_b N sodium hydroxide required to dilute it to have 0.1N silver nitrate solution = $\frac{100 \times 0.1}{N_b}$ = 'X' ml of given solution</p> 8. To check the normality of the final silver nitrate solution, again titrate it against 0.1 N sodium chloride and if the volume of sodium chloride consumed is equal to the volume of silver nitrate taken, then the solution is standardized one.

	9. For preparation of other concentration of silver nitrate, the solution can be prepared by weighing appropriate quantity (weight required in g = Equivalent weight x Normality to be prepared x Volume to be prepared in lit) and can be standardized using equivalent concentration of sodium chloride.
Reference	IS 2316: 1990 (Reaffirmed year 2001). Methods of preparation of standard solutions for colorimetric and volumetric analysis. Bureau of Indian Standards, New Delhi.
Approved by	Scientific Panel on Methods of Sampling and Analysis