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Food Safety and Standards Authority of India

(A statutory Authority established under the Food Safety and Standards Act, 2006)

(Quality Assurance Division)

FDA Bhawan, Kotla Road, New Delhi – 110002

Dated, the22nd June, 2021

ORDER

Subject: Revised FSSAI Manual of Methods of Analysis of Foods – reg.

Following Revised FSSAI Manual of Methods of Analysis of Foods have been approved by the Food Authority in its 33rd meeting held on 23.03.2021 and are enclosed herewith.

(i) Oils and Fats
(ii) Spices, Herbs and Condiments

2. The manuals shall be used by the laboratories with immediate effect. It supersedes the earlier manual on 'Oils and Fats' and 'Spices and condiments' issued vide Office Order No. 1-90/FSSAI/SP (MS&A)/2009 dated 25.05.2016.

3. Since the process of updation of test methods is dynamic, any changes happening from time to time will be notified separately. Queries/concerns, if any, may be forwarded to *email: sp-sampling@fssai.gov.in, dinesh.k@fssai.gov.in*

Encl: as above

(Harinder Singh Oberoi)
Advisor (QA)

To:

1. All FSSAI Notified Laboratories
2. All State Food Testing Laboratories

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

Inspiring Trust, Assuring Safe & Nutritious Food

Ministry of Health and Family Welfare, Government of India

MANUAL OF METHODS OF ANALYSIS OF FOODS

OILS AND FATS

PREFACE

Food safety requires an assurance that food will not cause any harm to the consumer, when it is prepared and/or consumed according to its intended use. There is a significant challenge in ensuring food safety to protect public health. Safeguarding food safety in today's complex world is a formidable task and is possible only with an intensive effort of all the stakeholders including regulatory authorities, industry and consumers.

The FSSAI Manual of Methods for Analysis of Oils and Fats is principally intended to provide unified, up-to-date testing methods for regulatory compliance. The manual brings together testing methodologies approved by FSSAI for use in surveillance and implementing the regulatory program. The objective here is to adopt "One Parameter - One Method" approach. These methods are dynamic and will be constantly updated, commensurate with the latest technological advancements in food analysis. The FSSAI notified laboratories shall use these testing methods only for analyzing samples under the Food Safety and Standards Act, 2006 and Food Safety and Standards Regulations, 2011.

Any suggestions/feedback from the stakeholders, which will contribute towards updating the manuals from time to time are welcome.

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Sincere thanks to the Panel, Chairman for their valuable guidance and encouragement and the Secretariat of this panel who have extended their support during this revision process.

Deepest appreciation to the Chairperson, FSSAI and CEO, FSSAI for their cooperation, support and constant encouragement without which the work would not have seen the light of day.

June 2021

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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.

MANUAL FOR ANALYSIS OF OILS AND FATS

Oils and fats are important parts of human diet and more than 90 percent of the world production from vegetable, animal and marine sources is used as food or as an ingredient in food products. Oils and fats are a rich source of dietary energy and contain more than twice the caloric value of equivalent amount of sugar. Their functional and textural characteristics contribute to the flavour and palatability of natural and prepared foods. They contain certain fatty acids which play an important role in nutrition and are also carriers of fat soluble vitamins.

The methods described in this manual are applicable for evaluating quality parameters such as acid value, fatty acid composition etc. For analytical methods related to heavy metal etc. the analyst should refer the relevant FSSAI Manual.

1.0 TYPES OF OILS AND FATS

Standards for 27 vegetable oils are prescribed in Section 2.2 of Food Safety and Standards (Food Product Standards and Food Additives) Regulations, 2011. Standards have also been laid down for Cocoa butter, Refined Salseed fat, Mango Kernel fat, Phulwara fat, Interesterified fat, Vanaspati, Table Margarine and Bakery / Industrial Margarine. Animal fats include Mutton /Goat fat and Lard.

2.0 GENERAL GLASSWARE AND APPARATUS

1. Beakers (different sizes)
2. Conical flasks with and without lids (different sizes)
3. Round bottom flasks (different sizes)
4. Standard volumetric flasks (different sizes)
5. Pipettes (different sizes)
6. Burettes(different sizes)
7. Measuring cylinders (different sizes)
8. Buchner funnels (different sizes)
9. Air condensers
10. Water condensers
11. Distillation heads
12. Receiving adapters
13. Ground glass joints
14. Mojonnier flask
15. Thermometers (different minimum and maximum temperatures in centigrade degrees)
16. Wash bottles (different sizes)
17. Separating funnels (different sizes)
18. Petri dishes (different sizes)

19. Weighing balances (upto milligram)
20. Weighing balances (upto gram)
21. Air Oven
22. Water bath temperature regulated
23. Hot plate magnetic stirrer
24. Falcon tubes (different sizes), Eppendorf microcentrifuge tubes (different size), GC-Vials, HPLC vials,
25. Desiccators
26. Whatman filter papers (different numbers)

All the above said apparatus and glassware needs to be calibrated periodically. Thermometer, oven, water bath etc. should be checked against a standard calibration certified by National Physical Laboratory, New Delhi or any other NABL approved Institution.

3.0 SAMPLE PREPARATION

Liquid Oils

Use clear sediment free liquid directly after inverting container several times. If liquid sample contains sediment release all sediment from walls of container and distribute uniformly throughout the oil for determination of moisture. For determinations in which results might be affected by possible presence of water (e. g iodine value) dry sample by adding anhydrous Sodium Sulphate in the proportion of 1 - 2 g per 10 g sample and hold it in oven at 50°C. Stir vigorously and filter to obtain clear filtrate.

Solid and semisolid Samples

Soften sample if necessary, by gently heating taking care not to melt it. When soft enough mix thoroughly for determination of moisture and volatile matter. For other determinations, melt in drying oven at a temperature at least 10°C above the melting point. If clear, proceed directly. If turbid or contains sediment filter test sample inside oven. For determinations in which results might be affected by possible presence of water (e.g. iodine value) dry sample by adding anhydrous Sodium Sulphate in the proportion of 1-2 g per 10 g sample and hold (keep) it in oven at 50°C. Stir vigorously and filter to obtain clear filtrate. To retard rancidity keep oils and fats in cool place and protect from light and air.

(Ref: - AOAC 17th edn, 2000. Official method 981.11 Oils and Fats – Preparation of test sample)

	Determination of Moisture Content – Air Oven Method		
Method No.	FSSAI 02.001:2021	**Revision No. & Date**	0.0
Scope	Water / moisture present in oil / fat sample is estimated.		
Caution	Phosphorus pentoxide - harmful if swallowed or inhaled. Fumes cause irritation to eyes and respiratory tract. Water reactive. Reacts violently with water to generate heat and phosphoric acid		
Principle	Moisture content of oils and fats is the loss in mass of the sample on heating at 105 ± 1 °C under operating conditions specified.		
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Metal dishes 7 – 8 cm diameter and 2 - 3 cm deep provided with tight fitting slip on covers. 3. Weighing Balance		
Materials and Reagents	1. Oils / Fats 2. Phosphorus pentoxide		
Sample Preparation	Refer 3.0 at page no. 2		
Method of analysis	1. Weigh in a previously dried and tared dish about 5 – 10 g of oil or fat, which has been thoroughly mixed by stirring. 2. Loosen the lid of the dish and heat, in an oven at 105±1 °C for 1 h. 3. Remove the dish from the oven and close the lid. 4. Cool in a desiccator containing phosphorus pentoxide or equivalent desiccant and weigh. 5. Heat in the oven for a further period of 1 h, cool and weigh. 6. Repeat this process until change in weight between two successive observations does not exceed 1 mg. 7. Carry out the determination in duplicate.		
Calculation with units of expression	Moisture and Volatile matter percentage = $\frac{W1 \times 100}{W}$ Where, W1 = Loss in weight (g) of the material on drying W = Weight in g of the material taken for test		
Reference	1. AOAC 17th edn., 2000, Official method 926.12, 2. ISI Hand book of Food Analysis (Part XIII) – 1984, page 62		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Specific Gravity		
Method No.	FSSAI 02.002:2021	**Revision No. & Date**	0.0
Scope	Specific gravity varies and depends on density of oil.		
Caution	Chromic Acid can cause reproductive damage. Handle with extreme caution. Chromic Acid is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Breathing Chromic Acid can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath.		
Principle	Specific gravity is the ratio of the density of a substance to the density of a reference substance (water); equivalently, it is the ratio of the mass of a substance to the mass of a reference substance (water) for the same given volume.		
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Pycnometer fitted with a thermometer of suitable range (with 0.1 or 0.2 °C subdivision) or a density bottle. 3. Weighing Balance 4. Water bath maintained at 30 ± 2.0 °C.		
Materials and Reagents	Oils / Fats		
Preparation of reagents	1. The thermometer should be checked against a standard thermometer calibrated and certified by National Physical Laboratory, New Delhi or any other NABL approved institution. **Standardization of Pycnometer** 2. Carefully clean the pycnometer by filling with Chromic acid cleaning solution and letting it stand for several hours. 3. Empty pycnometer and rinse thoroughly with water, fill with recently boiled water, previously cooled to about 20 °C and place in constant temperature water bath held at 30 °C. 4. After 30 min adjust water level to proper point on pycnometer and stopper, remove from bath, wipe dry with chem wipes/clean cloth or towel and weigh.		
Sample Preparation	1. Melt sample if necessary. Filter through a filter paper to remove any impurities and the last traces of moisture. 2. Make sure that the sample is completely dry. 3. Cool the sample to 30 °C or ambient temperature desired for determination. Refer 3.0 at page no. 2		
Method of analysis	1. Fill the dry pycnometer with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. 2. Insert the stopper, immerse in water bath at 30±2.0 °C and hold for 30 min. 3. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it		

	thoroughly. 4. Remove the cap of the side arm and quickly weigh ensuring that the temperature does not fall below 30 °C.
Calculation with units of expression	Specific Gravity at 30 ° C (g/mL) $= \frac{A-B}{C-B}$ Where, A = weight in g of specific gravity bottle with oil at 30 °C B = weight in g of specific gravity bottle at 30 °C C = weight in g of specific gravity bottle with water at 30 °C
Reference	1. AOAC 17th edn., 2000, Official method 920.212 Specific gravity (Apparent) of Oils, Pycnometer method. 2. ISI Hand book of Food Analysis (Part XIII) 1984, page 72
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Refractive Index**		
Method No.	FSSAI 02.003:2021	**Revision No. & Date**	0.0
Scope	Refractive index varies with temperature and wavelength. Significance: Refractive index of oils increases with the increase in unsaturation and also chain length of fatty acids.		
Principle	The ratio of velocity of light in vacuum to the velocity of light in the oil or fat; more generally, expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wave length (usually 589.3 nm, the mean of D lines of Sodium) passes from air into the oil or fat. Measurement of the refractive index of the sample is done by means of a suitable refractometer.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Butyro Refractometer or Abbe Refractometer <u>Abbes Refractometer</u> (i) Open double prism with the help of the screw head and place a drop of oil on the prism. (ii) Close prisms firmly by tightening screw heads. (iii) As refractive index is greatly affected by temperature, the temperature of the refractometer should be controlled to within ± 0.1 °C and for this purpose it should be provided with a thermostatically controlled water bath and a motor driven pump to circulate water through the instrument. <u>Butyro refractometer</u> (i) Its reading can be converted to refractive index with the help of the table. (ii) Light Source -If the refractometer is equipped with a compensator, a tungsten lamp or day light may be used. (iii) Otherwise a monochromatic light such as sodium vapour lamp (589.3 nm) may be used.		
Materials and reagents	Oil / Fat		
Preparation of reagents / Calibration of apparatus	1. The instrument is calibrated with a glass prism of known refractive index (an optical contact with the prism being made by a drop of a bromonaphthalene) or by using distilled water which has refractive index of 1.3330 at 20.0 °C and 1.3306 at 40.0 °C, the usual temperature of taking readings.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of analysis	1. Melt the sample if it is not already liquid and filter through a filter paper containing anhydrous Sodium Sulphate in the proportion of 1 - 2 g per 10 g sample previously heated in oven at 50 °C, to remove impurities and traces of moisture. 2. Make sure sample is completely dry.		

	3. Circulate stream of water through the instrument. 4. Adjust the temperature of the refractometer to the desired temperature. 5. Ensure that the prisms are clean and dry. 6. Place a few drops of the sample on the prism. 7. Close the prisms and allow standing for 1-2 min. 8. Adjust the instrument and lighting to obtain the most distinct reading possible and determining the refractive index or butyro-refractometer number as the case may be. 9. After recording the measurement, wipe the prism with tissue to remove the oil and wipe with isoproponal and pet ether to clean the prism for next sample analysis.
Calculation with units of expression	**Temperature correction:** Determine refractive index at the specified temperature. If temperature correction is necessary use following formula: $R = R^{1} + K (T^{1} – T)$ Where, R = Reading of the refractometer reduced to the specified temperature T °C R^{1} = Reading at T^{1} °C K = constant 0.000365 for fats and 0.000385 for oils (If Abbe Refractometer is used) or = 0.55 for fats and 0.58 for oils (if Butyro-refractometer is used) T^{1} = temperature at which the reading R^{1} is taken and T = specified temperature (generally 40 °C.)
Reference	1. AOAC 17th edn, 2000, Official method 921.08 – Index of refraction of oils and fats. 2. ISI Handbook of Food analysis (Part XIII) – 1984, page 70) Table for conversion of B.R. readings to Refractive Index
Approved by	Scientific Panel on Methods of Sampling and Analysis

Table 1:	**Butyro refractometer reading and indices of refraction (n_D)[a]**									
Fourth Decimal of n_D										
nD	0	1	2	3	4	5	6	7	8	9
Butyro Scale Readings										
1.422	0.0	0.1	0.2	0.4	0.5	0.6	0.7	0.9	1.0	1.1
1.423	1.2	1.4	1.5	1.6	1.7	1.9	2.0	2.1	2.2	2.4
1.424	2.5	2.6	2.7	2.8	3.0	3.1	3.2	3.3	3.5	3.6
1.425	3.7	3.8	4.0	4.1	4.2	4.3	4.5	4.6	4.7	4.8
1.426	5.0	5.1	5.2	5.4	5.5	5.6	5.7	5.9	6.0	6.1
1.427	6.2	6.4	6.5	6.6	6.8	6.9	7.0	7.1	7.2	7.4
1.428	7.5	7.6	7.7	7.9	8.0	8.1	8.2	8.4	8.5	8.6
1.429	8.7	8.9	9.0	9.1	9.2	9.4	9.5	9.6	9.8	9.9
1.430	10.0	10.1	10.3	10.4	10.5	10.6	10.7	10.9	11.0	11.1
1.431	11.3	11.4	11.5	11.6	11.8	11.9	12.0	12.2	12.3	12.4
1.432	12.5	12.7	12.8	12.9	13.0	13.2	13.3	13.5	13.6	13.7
1.433	13.8	14.0	14.1	14.2	14.4	14.5	14.6	14.7	14.9	15.0
1.434	15.1	15.3	15.4	15.5	15.6	15.8	15.9	16.0	16.2	16.3
1.435	16.4	16.6	16.7	16.8	17.0	17.1	17.2	17.4	17.5	17.6
1.436	17.8	17.9	18.0	18.2	18.3	18.4	18.5	18.7	18.8	18.9
1.437	19.1	19.2	19.3	19.5	19.6	19.7	19.8	20.0	20.1	20.2
1.438	20.4	20.5	20.6	20.8	20.9	21.1	21.2	21.3	21.4	21.6
1.439	21.7	21.8	22.0	22.1	22.2	22.4	22.5	22.6	22.7	22.9
1.440	23.0	23.2	23.3	23.4	23.5	23.7	23.8	23.9	24.1	24.2
1.441	24.3	24.5	24.6	24.7	24.8	25.0	25.1	25.2	25.4	25.5
1.442	25.6	25.8	25.9	26.1	26.2	26.3	26.5	26.6	26.7	26.9
1.443	27.0	27.1	27.3	27.4	27.5	27.7	27.8	27.9	28.1	28.2
1.444	28.3	28.5	28.6	28.7	28.9	29.0	29.2	29.3	29.4	29.6
1.445	29.7	29.9	30.0	30.1	30.3	30.4	30.6	30.7	30.8	30.9
1.446	31.1	31.2	31.4	31.5	31.6	31.8	31.9	32.1	32.2	32.3

Table 1:	**Butyro refractometer reading and indices of refraction (n_D)[a]**									
Fourth Decimal of n_D										
nD	0	1	2	3	4	5	6	7	8	9
Butyro Scale Readings										
1.447	32.5	32.6	32.8	32.9	33.0	33.2	33.3	33.5	33.6	33.7
1.448	33.9	34.0	34.2	34.3	34.4	34.6	34.7	34.9	35.0	35.1
1.449	35.3	35.4	35.6	35.7	35.8	36.0	36.1	36.3	36.4	36.5
1.450	36.7	36.8	37.0	37.1	37.2	37.4	37.5	37.7	37.8	37.9
1.451	38.1	38.2	38.3	38.5	38.6	38.7	38.9	39.0	39.2	39.3
1.452	39.5	39.6	39.7	39.9	40.0	40.1	40.3	40.4	40.6	40.7
1.453	40.9	41.0	41.1	41.3	41.4	41.5	41.7	41.8	42.0	42.1
1.454	42.3	42.4	42.5	42.7	42.8	43.0	43.1	43.3	43.4	43.6
1.455	43.7	43.9	44.0	44.2	44.3	44.4	44.6	44.7	44.9	45.0
1.456	45.2	45.3	45.5	45.6	45.7	45.9	46.0	46.2	46.3	46.4
1.457	46.6	46.7	46.9	47.0	47.2	47.3	47.5	47.6	47.7	47.9
1.458	48.0	48.2	48.3	48.5	48.6	48.8	48.9	49.1	49.2	49.4
1.459	49.5	49.7	49.8	50.0	50.1	50.2	50.4	50.5	50.7	50.8
1.460	51.0	51.1	51.3	51.4	51.6	51.7	51.9	52.0	52.2	52.3
1.461	52.5	52.7	52.8	53.0	53.1	53.3	53.4	53.6	53.7	53.9
1.462	54.0	54.2	54.3	54.5	54.6	54.8	55.0	55.1	55.3	55.4
1.463	55.6	55.7	55.9	56.0	56.2	56.3	56.5	56.6	56.8	56.9
1.464	57.1	57.3	57.4	57.6	57.7	57.9	58.0	58.2	58.3	58.5
1.465	58.6	58.8	58.9	59.1	59.2	59.4	59.5	59.7	59.8	60.0
1.466	60.2	60.3	60.5	60.6	60.8	60.9	61.1	61.2	61.4	61.5
1.467	61.7	61.8	62.0	62.2	62.3	62.5	62.6	62.8	62.9	63.1
1.468	63.2	63.4	63.5	63.7	63.8	64.0	64.2	64.3	64.5	64.7
1.469	64.8	65.0	65.1	65.3	65.4	65.6	65.7	65.9	66.1	66.2
1.470	66.4	66.5	66.7	66.8	67.0	67.2	67.3	67.5	67.7	67.8
1.471	68.0	68.1	68.3	68.4	68.6	68.7	68.9	69.1	69.2	69.4

Table 1:	**Butyro refractometer reading and indices of refraction (n_D)[a]**									
Fourth Decimal of n_D										
nD	0	1	2	3	4	5	6	7	8	9
Butyro Scale Readings										
1.472	69.5	69.7	69.9	70.0	70.2	70.3	70.5	70.7	70.8	71.0
1.473	71.1	71.3	71.4	71.6	71.8	71.9	72.1	72.2	72.4	72.5
1.474	72.7	72.9	73.0	73.2	73.3	73.5	73.7	73.8	74.0	74.1
1.475	74.3	74.5	74.6	74.8	75.0	75.1	75.3	75.5	75.6	75.8
1.476	76.0	76.1	76.3	76.5	76.7	76.8	77.0	77.2	77.3	77.5
1.477	77.7	77.9	78.1	78.2	78.4	78.6	78.7	78.9	79.1	79.2
1.478	79.4	79.6	79.8	80.0	80.1	80.3	80.5	80.6	80.8	81.0
1.479	81.2	81.3	81.5	81.7	81.9	82.0	82.2	82.4	82.5	82.7
1.480	82.9	83.1	83.2	83.4	83.6	83.8	83.9	84.1	84.3	84.5
1.481	84.6	84.8	85.0	85.2	85.3	85.5	85.7	85.9	86.0	86.2
1.482	86.4	86.6	86.7	86.9	87.1	87.3	87.5	87.6	87.7	88.0
1.483	88.2	88.3	88.5	88.7	88.9	89.1	89.2	89.4	89.6	89.8
1.484	90.0	90.2	90.3	90.5	90.7	90.9	91.2	91.2	91.4	91.6
1.485	91.8	92.0	92.1	92.3	92.5	92.7	92.9	93.0	93.2	93.4
1.486	93.6	93.8	94.0	94.1	94.3	94.5	94.7	94.8	95.0	95.2
1.487	95.4	95.6	95.8	96.0	96.1	96.3	96.5	96.7	96.9	97.0
1.488	97.2	97.4	97.6	97.8	98.0	98.1	98.3	98.5	98.7	98.9
1.489	99.1	99.2	99.4	99.6	99.8	100.0	…	….	…	….

	Determination of Flash Point : Pensky Marten (closed cup) Method		
Method No.	FSSAI 02.004:2021	**Revision No. & Date**	0.0
Scope	Flash point is the lowest temperature at which a liquid can form an ignitable mixture in air near the surface of the liquid. The method determines the temperature at which the sample will flash, when a test flame is applied under the conditions specified for the test.		
Principle	The sample is heated in a test cup at a slow and constant rate with continual stirring. A small test flame is directed into the cup at regular intervals with simultaneous interruption of stirring. The flash point is taken as the lowest temperature at which the application of the test flame causes the vapour above the sample to ignite momentarily.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Pensky-Martens closed cup apparatus with thermometer.		
Materials and reagents	Oils and Fats		
Sample Preparation	Refer 3.0 at page no. 2 1. Samples containing dissolved or free water may be dehydrated with Calcium chloride or by filtering through a suitable filter paper or a loose plug of dry absorbent cotton. 2. Warming the sample is permitted but it shall not be heated for prolonged periods or above the temperature of 16 °C below its expected flash point.		
Method of Analysis	1. Thoroughly clean and dry all parts of the cup and its accessories before starting the test, being sure to remove any solvent which had been used to clean the apparatus. 2. Support the tester on a level steady table. 3. Fill the cup with the oil to be tested up to the level indicated by the filling mark. 4. Place the lid on the cup and properly engage the heating devices. Insert the thermometer, light the test flame and adjust it to 4.0 mm in diameter. 5. Heat the sample so that the temperature increase is about 5 to 6 °C per min. 6. During the heating, turn the stirring device from one to two revolutions per second. 7. Apply the test flame when the temperature of the sample is a whole number not higher than 17 °C below the flash point. 8. At every 5 °C rise in temperature, discontinue stirring and apply the test flame by opening the device which controls the shutter and lowers the test flame into the shutter opening. 9. Lower the test flame in for 0.5 sec and quickly return to the raised position. Do not stir the sample while applying the test flame. 10. As soon as the test flame has been returned to the raised position, resume stirring. 11. The flash point is the temperature indicated by the thermometer at the time of the flame application that causes a distinct flash in the		

	interior of the cup.
Calculation with units of expression	Flash point of oil or fat is expressed as degree of Celsius (^{o}C)
Reference	IS 1448 – 1970 Methods of test for petroleum and its products (P: 21) Flash Point (Closed) by Pensky Martin apparatus
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Color		
Method No.	FSSAI 02.005:2021	**Revision No. & Date**	0.0
Scope	Color measurement in the oils and fats industry is an essential part of the refining process. It is a means of assessing when the desired color has been reached and when the refining can be halted.		
Principle	The method determines the color of oils by comparison with Lovibond glasses of known color characteristics. The color is expressed as the sum total of the yellow and red slides used to match the color of the oil in a cell of the specified size in the Lovibond Tintometer.		
Apparatus/ Instruments	1. General Glass ware and apparatus (Refer 2.0 at page no. 1) 2. Lovibond Tintometer 3. Glass cells (cell size 0.25 inch, 0.5 inch.1.0 inch, 5.25 inch or 1.0 cm, 2.0 cm, 5.0 cm as required)		
Materials and Reagents	Oils / Fats		
Sample Preparation	Melt the sample if it is not already liquid and filter the oil through a filter paper to remove any impurities and traces of moisture. Make sure sample is absolutely clear and free from turbidity. Refer 3.0 at page no. 2		
Method of analysis	1. Clean the glass cell of desired size with carbon tetrachloride and allow it to dry. 2. Fill it with the oil and place the cell in position in the tintometer. 3. Match the color with sliding red, yellow and blue colors.		
Calculation with units of expression	Report the color of the oil in terms of Lovibond units as follows: Color reading = (a Y + 5 b R) or (a Y + 10 b R) in (* cell) Where, a = sum total of the various yellow slides (Y) used b = sum total of the various red (R) slides used Y + 5R is the mode of expressing the color of light colored oils; and Y + 10 R is for the dark-colored oils Although the yellow and red slides required to match the color shade of an oil in a tintometer are assessed separately, it is found that to a certain extent these slides are mutually compensatory.		
Inference (Qualitative Analysis)	Consequently different workers may report different values for the yellow and red units for the same oil and the same workers may report different values for the yellow and red units for the oil examined at different times. To obviate such personal errors a composite factor is used for checking the color comprising the sum total of the yellow(Y) units and 5 or 10 times the total of red units as specified for the oil or fat.		
Reference	1. ISI Hand book of Food Analysis (Part XIII) – 1984 page 75. 2. IS 548 (Part 1) – 1964, Methods of sampling and test for Oils and Fats.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Slip Melting Point of Fat		
Method No.	FSSAI 02.006:2021	**Revision No. & Date**	0.0
Scope	Oils and fats are chiefly mixtures of triglycerides. They do not exhibit either a definite or sharp melting point. Therefore, the melting point does not imply the same characteristics that it does with pure crystalline substances. Fats pass through a stage of gradual softening before they become completely liquid. The melting point is therefore defined by the specific conditions of the method by which it is determined.		
Principle	**Open-tube Capillary-Slip Method** The melting point is the temperature at which the oil or fat softens or becomes sufficiently fluid to slip or run as determined by the open-tube capillary-slip method.		
Apparatus / Instruments	1. General glass ware and apparatus (Refer 2.0 at page no. 1) 2. Melting point tubes -thin walled with uniform bore capillary glass tubes open at both ends with following dimensions: Length 50 to 80 mm Inside diameter 1.0mm Outside diameter 2.0 mm 3. Thermometer with 0.2 °C sub-divisions with a suitable range. The thermometer should be checked against a standard thermometer that has been calibrated and certified by National Physical Laboratory, New Delhi or any other laboratory approved for calibration of instruments. 4. Beaker with a side tube heating arrangement – Thiele melting point tube may be used. Alternatively, a melting point apparatus may also be used. 5. Heat source: Gas burner or Spirit Lamp or electric hot plate with rheostat control.		
Materials and Reagents	Fats		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Melt the sample and filter it through a filter paper to remove any impurities and last traces of moisture. 2. Make sure that the sample is absolutely dry. Mix the sample thoroughly. 3. Introduce a capillary tube into the molten sample, so that a column of the sample, about 10 mm long, is sucked into the tube. 4. Dip atleast 3 clean capillary tubes in the completely liquid sample so that the sample rises about 10 mm high in tubes. 5. Chill the sample at once by holding the ends of the tubes that contain the sample against a piece of ice until the fat solidifies. 6. Place the tube in a small beaker and hold it in a refrigerator at 4 °C to 10 °C for 16 h. 7. Remove the tube from the refrigerator and attach with a rubber band to the thermometer bulb, so that the lower end of the capillary tube		

	and the thermometer bulb are at the same level. 8. Suspend the thermometer in 600 mL beaker of clear distilled water. The bottom of thermometer is immersed in the water to the immersion mark. 9. Take water at 10 °C in the 'Thiele' tube and immerse the thermometer with the capillary tube containing the sample of fat. Gradually increase the temperature by heating at the side-tube of the Thiele Tube at the rate of 2 °C per min, till the temperature reaches 25 °C, and thereafter at the rate of 0.5 °C per min. 10. Note the temperature of the water when the sample column begins to rise in the capillary tube.
Inference (Qualitative Analysis)	Report the average of two such separate determinations as the melting point, provided that the readings do not differ by more than 0.5 °C.
Reference	1. ISI Handbook of Food Analysis (Part XIII) – 1984, page 68. 2. IS: 548 (Part 1) – 1964, Methods of Sampling and test for Oils and Fats page 33. 3. AOCS Official Method Cc 3-25 – Slip melting point-AOCS Standard Open Tube Melting Point.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Saponification Value		
Method No.	FSSAI 02.007:2021	**Revision No. & Date**	0.0
Scope	The saponification value is the number of mg of Potassium hydroxide required to saponify 1 g of oil/fat.		
Caution	1. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Sodium Carbonate: Eye contact can cause permanent corneal injury and possible burns. Avoid ingestion or inhalation of dust. Due to these potential hazards, sodium carbonate should be handled with care.		
Principle	The oil sample is saponified by refluxing with a known excess of alcoholic Potassium hydroxide solution. The alkali required for saponification is determined by titrating the excess Potassium hydroxide with standard hydrochloric acid. **Importance:** The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa.		
Apparatus/ Instruments	1. General Glass ware and apparatus (Refer 2.0 at page no. 1) 2. 250 mL capacity conical flask with ground glass joints. 3. 1 m long air condenser, or reflux condenser (65 cm minimum in length) to fit the flask. 4. Hot water bath or electric hot plate fitted with thermostat. 5. 1000 mL volumetric flask / stoppered flask. 6. Weighing flask 7. Balance		
Materials and Reagents	1. Aldehyde free alcohol 2. Potassium hydroxide 3. Distilled water 4. Phenolphthalein indicator 5. Hydrochloric acid 6. Anhydrous standard Sodium / Potassium carbonate		
Preparation of reagents	1. Alcoholic Potassium hydroxide Solution - 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 and keep in a bottle closed tight with a cork or rubber stopper. 2. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein in 100 mL rectified spirit. 3. Standard hydrochloric acid: approximately 0.5N (Standardized against anhydrous sodium / potassium carbonate)
Sample Preparation	Refer 3.0 at page no. 2
Method of analysis	1. Melt the sample if it is not already liquid and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry. 2. Mix the sample thoroughly and weigh about 1.5 to 2.0 g of dry sample into a 250 mL Erlenmeyer flask. 3. Pipette 25 mL of the alcoholic Potassium hydroxide solution into the flask. Conduct a blank determination along with the sample. 4. Connect the sample and blank flasks with air condensers; keep on the water bath, gently and steadily boiling until saponification is complete, indicated by absence of any oily matter and the appearance of a clear solution. 5. Clarity may be achieved within one hour of boiling. 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. 6. The excess Potassium hydroxide is determined by titration with 0.5N hydrochloric acid, using about 1.0 mL phenolphthalein indicator.
Calculation with units of expression	Saponification Value = $\frac{56.1\times(B-S)\times N}{W}$ Where, B = Volume in mL of standard hydrochloric acid required for the blank. S = Volume in mL of standard hydrochloric acid required for the sample N = Normality of the standard hydrochloric acid and W = Weight in g of the oil/fat taken for the test. Units: mg of KOH/1 g oil or fat Note: - When titrating oils and fats, which give dark colored soap solution the observation of the end point of titration may be facilitated either (a) by using thymolphthalein or alkali blue 6B in place of phenolphthalein or (b) by shaking 1mL of 0.1% (w/v) solution of methylene blue in water to each 100mL of phenolphthalein indicator solution before the titration.
Reference	1. AOAC 17th edn, 2000, Official method 920.160 Saponification number of oils and fats 2. IUPAC 2. 202 3. ISI Handbook of Food Analysis (Part XIII) 1984, page 78) 4. IS: 323-1959 Specification for Rectified Spirit (*Revised*)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Unsaponifiable Matter		
Method No.	FSSAI 02.008:2021	**Revision No. & Date**	0.0
Scope	Unsaponifiable matter is defined as the substances soluble in the oil, which after saponification are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins, and hydrocarbons as well as any foreign organic matter non-volatile at 100 °C e.g. (mineral oil).		
Caution	1. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 2. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 3. Potassium hydroxide: It is corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 4. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	Light Petroleum or diethyl ether is used as a solvent but in most cases results will differ according to the solvent selected and generally the use of diethyl ether will give a higher value.		
Apparatus / Instruments	1. General Glass ware and apparatus (Refer 2.0 at page no. 1) 2. Flat bottom flask or conical flask with a ground glass joint, 250 mL capacity 3. Air condenser 1 meter long to fit the flask 4. Separating funnel, 500 mL capacity 5. Weighing balance-The weighing balance should be accurately calibrated to measure 10 mg of sample on a tare weigh of 100 g.		
Materials and Reagents	1. Potassium hydroxide 2. Ethyl alcohol (aldehyde free) 3. Ethyl alcohol: Ninety-five percent 4. Phenolphthalein 5. Petroleum ether (40 – 60 °C): Analytical reagent grade 6. Sodium hydroxide 7. Acetone: Analytical reagent grade 8. Anhydrous sodium sulphate		
Preparation of reagents	1. Alcoholic Potassium hydroxide solution: Dissolve 7 to 8 g of Potassium hydroxide in an equal quantity of distilled water and add		

	sufficient aldehyde free ethyl alcohol and make up to 100 mL. 2. Phenolphthalein indicator solution: Dissolve one gram of phenolphthalein in 100 mL of ethyl alcohol. 3. Aqueous alcohol: 10% of ethyl alcohol in water 4. Standard sodium hydroxide solution: Approximately 0.02N
Sample Preparation	Refer 3.0 at page no. 2
Method of analysis	1. Weigh accurately 5 g of well mixed oil/fat sample into a 250 mL conical flask. Add 50 mL of alcoholic Potassium hydroxide solution. 2. Boil the content gently but steadily under reflux air condenser for one hour or until the saponification is complete (complete saponification gives a homogeneous and transparent medium). Take care to avoid loss of ethyl alcohol during the saponification. 3. Wash the condenser with about 10 mL of ethyl alcohol. Transfer the saponified mixture while still warm to a separating funnel, wash the saponification flask first with some ethyl alcohol and then with cold water, using a total of 50 mL of water to rinse the flask. 4. Cool to 20 to 25 °C. Add to the flask 50 mL of petroleum ether, insert the stopper and shake vigorously, and allow the layers to separate until two distinct layers are obtained. 5. Transfer the lower soap layer into another separating funnel and repeat the ether extraction 3 times, using 50 mL portions of petroleum ether for each extraction. If any emulsion is formed, add a small quantity of ethyl alcohol or alcoholic Potassium hydroxide solution. 6. Some oils high in unsaponifiable matter, e.g., marine oils, may require more than three extractions to completely remove Unsaponifiable matter. In that case repeat the ether extraction 3 times more, using 50 mL portions of petroleum ether for each extraction. 7. Collect all the ether extracts in a separating funnel. Wash the combined ether extract three times with 25 mL portions of aqueous alcohol followed by washing with 25 mL portions of distilled water to ensure ether extract is free of alkali (washing are no longer alkaline to phenolphthalein). 8. Transfer washed ether extract to 250 mL beaker containing a few pieces of pumice stone, rinse separator with ether, and add rinsing to main solution. 9. Evaporate to about 5 mL and transfer quantitatively using several portions of ether to a previously dried and weighed 50 mL Erlenmeyer flask. 10. Evaporate ether by placing on a water bath. When all ether has been removed add 2-3 mL acetone and while heating on steam or water bath completely remove solvent under a gentle air. 11. To remove last traces of ether, dry at 100 °C for 30 min till constant weight. Note the weight. Dissolve residue in 50 mL of warm ethanol, which has been neutralised to a phenolphthalein end point. Titrate with 0.02N Sodium hydroxide.

Calculation with units of expression	Weight in g of the free fatty acids in the extract as oleic acid = 0.282 V×N Where, V = Volume in mL of standard sodium hydroxide solution N = Normality of standard sodium hydroxide solution Unsaponifiable matter percentage = $\frac{100\times(A-B)}{W}$ Where, A = Weight of the residue in g B = Weight of free fatty acids in the extract in g W = Weight of the sample in g
Reference	1. FAO Manual of Food quality control 14/8, page 261. 2. ISI Handbook of Food Analysis (Part XIII)-1984, page 67 3. AOAC 17th edn, 2000, Official method 933.08, Residue (unsaponifiable) of oils and fats.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Acid Value		
Method No.	FSSAI 02.009:2021	**Revision No. & Date**	0.0
Scope	The acid value is defined as the number of milligrams of Potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid, lauric, ricinoleic and palmitic acids.		
Caution	1. Potassium hydroxide: It is corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard Potassium hydroxide/sodium hydroxide solution. The value is a measure of the amount of fatty acids, which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lipolytic enzyme lipase.		
Apparatus / Instruments	1. General Glass ware and apparatus (Refer 2.0 at page no. 1). 2. Ambered colored bottle. 3. Brown glass bottle		
Materials and Reagents	1. Oils and fats 2. Phenolphthalein indicator 3. Ethyl alcohol 4. Alkali Blue 6B indicator 5. Potassium hydroxide or sodium hydroxide solution		
Preparation of reagents	1. Phenolphthalein indicator solution: - Dissolve one gram of phenolphthalein in 100 mL of ethyl alcohol. 2. Alkali Blue 6B indicator solution: When testing rice bran oil or rice bran oil based blended oils or fats, which give dark colored soap solution, the observation of the end point of the titration may be facilitated, by using Alkali Blue 6B in place of Phenolphthalein. 3. Preparation: (2%) Extract 2 g of alkali blue 6B with rectified spirit in a Soxhlet apparatus at reflux temperature. Filter the solution if necessary and dilute to 100 mL with rectified spirit. Alkali blue 6B indicator to be stored in closed Ambered colored bottle to avoid oxidation of dye. 4. Ethyl alcohol: (i). Ninety-five percent alcohol or rectified spirit neutral to phenolphthalein indicator. (ii). Ninety-five percent alcohol or rectified spirit neutral to Alkali blue		

<table>
<tr><td></td><td>6B indicator in case of rice bran oil or rice bran oil based blended oil or fats.
5. Standard aqueous Potassium hydroxide or sodium hydroxide solution 0.1 or 0.5 N. The solution should be colorless and stored in a brown glass bottle. For refined oils, the strength of the alkali should be fixed to 0.1 N.</td></tr>
<tr><td>Sample Preparation</td><td>Refer 3.0 at page no. 2</td></tr>
<tr><td>Method of Analysis</td><td>Mix the oil or melted fat thoroughly before weighing. The mass of the test sample shall be taken based on the color and expected acid value.
<table>
<tr><th>Expected Acid Value</th><th>Mass of Test portion(g)</th><th>Accuracy of weighing of test portion (g)</th></tr>
<tr><td><1</td><td>20</td><td>0.05</td></tr>
<tr><td>1 to 4</td><td>10</td><td>0.02</td></tr>
<tr><td>4 to 15</td><td>2.5</td><td>0.01</td></tr>
<tr><td>15 to 75</td><td>0.5</td><td>0.001</td></tr>
<tr><td>>75</td><td>0.1</td><td>0.0002</td></tr>
</table>
Weigh accurately appropriate amount of the cooled oil sample as mentioned in the above table in a 250 mL conical flask.
Add 50 mL of freshly neutralised hot ethyl alcohol and about one ml of phenolphthalein indicator solution. In case of rice bran oil or RBO based blends, add about 1 mL of Alkali blue indicator.
Heat the mixture for about fifteen min in water bath (75-80 °C)
In case of Rice bran oil or RBO based blended oils or fats, add 1mL of Alkali blue indicator after heating.
Titrate while hot against standard alkali solution shaking vigorously during the titration.
End point using phenolphthalein indicator shall be from colorless to light pink (Persisting for 15 sec.).
End point using Alkali blue 6B indicator shall be disappearance of blue color which developed during addition of indicator.

Note: Noting burette reading after “obtaining dark pink color OR Orangish red” as end point should be avoided as it will lead to erroneous result.
The weight of the oil/fat taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration does not exceed 10 mL.</td></tr>
<tr><td>Calculation with units of expression</td><td>Acid value = $\frac{56.1 \times V \times N}{W}$
Where,
V = Volume in mL of standard Potassium hydroxide or sodium hydroxide used
N = Normality of the Potassium hydroxide solution or Sodium hydroxide solution; and
W = Weight in g of the sample
Acid value = % fatty acid (as oleic) × 1.99</td></tr>
</table>

	The acidity is frequently expressed as the percentage of FFA in the sample. The percentage of FFA in most oils and fats is calculated on the basis of oleic acid; although in coconut oil and palm kernel oil it is often calculated as lauric acid, in castor oil in terms of ricinoleic acid and in palm oil in terms of palmitic acid. Free fatty acid as oleic acid % by weight = 28.2 ×V× N/W Free fatty acid as lauric acid % by weight = 20 ×V×N/W Free fatty acid as ricinoleic acid % by weight =29.8 ×V× N/W Free fatty acid as palmitic acid % by weight =25.6 ×V×N/W Note: Oryzanol has its own acidity and contributes to the measured FFA content when present in oil. FFA content determined by using phenolphthalein as the indicator needs to be corrected. The formula for calculating real FFA content is shown below. Real FFA = observed FFA (for phenolphthalein) – (% oryzanol in the oil) x 0.425 For determination of acid value in case of rice bran oil and blended oils containing rice bran oil, the correction factor provided above must be used to account for oryzanol's acidity or alkali blue may be used as an indicator for the titration which is most suitable.
Reference	1. ISI Handbook of Food Analysis (Part XIII)-1984 Page 67 2. IUPAC 2.201(1979) 3. IS: 548 (Part 1) – 1964, Methods of Sampling and Test for Oils and Fats 4. ISO 660:1996 Determination of acid value and acidity 5. AOAC 17th edn, 2000, Official method 940.28
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Iodine Value		
Method No.	FSSAI 02.010:2021	**Revision No. & Date**	0.0
Scope	The iodine value of an oil/fat is the number of grams of iodine absorbed by 100 g of the oil/fat, when determined by using Wijs solution.		
Caution	1. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 2. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Carbon tetrachloride: It is a highly toxic narcotic and central nervous system depressant causing possible unconsciousness, coma and death from respiratory failure. It causes permanent kidney and liver damage. It can be absorbed via the skin as well as by inhalation or ingestion. 4. Potassium iodide: Common side effects of Potassium Iodide include: Allergic reactions (skin rashes such as hives; swelling of various parts of the body such as the face, lips, tongue, throat, hands or feet; fever with joint pain, trouble breathing, speaking or swallowing, wheezing, or shortness of breath).		
Principle	The oil/fat sample taken in carbon tetrachloride is treated with a known excess of iodine monochloride solution in glacial acetic (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulfate solution Importance - The iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat.		
Apparatus / Instruments	1. General glass ware and apparatus (Refer 2.0 at page no. 1) 2. Erlenmeyer flasks 3. Brown glass bottles 4. Beakers 5. Burettes 6. Pipettes 7. Volumetric flasks		
Materials and Reagents	1. Potassium dichromate 2. Concentrated hydrochloric acid AR 3. Glacial acetic acid, free from ethanol 4. Carbon tetrachloride, analytical reagent grade 5. Iodine mono-chloride (ICl) 6. Potassium iodide (free from potassium iodate) 7. Starch		

	8. Mercuric iodide 9. Glacial acetic acid 10. Sodium thiosulphate 11. Resublimed Iodine 12. Dried chlorine (dried through H_2SO_4) 13. Saturated Cl – water
Preparation of reagents	1. Potassium iodide (free from potassium iodate) - 10% solution prepared fresh. 2. Starch solution - Mix 5 g of starch and 0.01 g of 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 the clear supernatant. 3. Wijs Iodine monochloride solution: (i) Dissolve 10 mL of iodine monochloride in about 1800 mL of glacial acetic acid and shake vigorously. (ii) Pipette 5 mL of Wijs 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 Wijs iodine solution by dissolving 13 g resublimed Iodine in 1000 mL acetic acid and pass in dried chlorine (dried through H_2SO_4) until original Sodium thiosulphate titre value of the solution is not quite doubled (characteristic color 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 Chlorine to bulk of solution and bring to desired titre by re-additions of reserved portion). (iii) Store in an amber colored bottle sealed with paraffin until ready for use. Wijs solutions are sensitive to temperature, moisture and light. Store in the dark below 30 °C. Determine I/Cl ratio as follows Iodine Content – Pipette 5 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL saturated Cl – water and some glass beads. Shake heat to boiling point and boil briskly for 10 min. Cool, add 30 mL H_2SO_4 (1+ 49) and 15 mL 15% Potassium iodide solution and titrate immediately with 0.1 N Sodium thiosulphate. (iv) Total Halogen content – Pipette 20 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL recently boiled and cooled water and 15 mL 15 % Potassium iodide solution. Titrate immediately with 0.1 N Sodium thiosulphate. I/Cl = 2 X / (3B – 2 X) where X = mL of 0.1 Sodium thiosulphate required for I content and B = mL required for total halogen content. I / Cl ratio must be 1.10±0.1 4. Standard sodium thiosulphate solution (0.1N) (i). Dissolve approximately24.8 g of sodium thiosulphate crystals ($Na_2S_2O_3.5H_2O$) in distilled water and make up to 1000 mL. (ii). 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

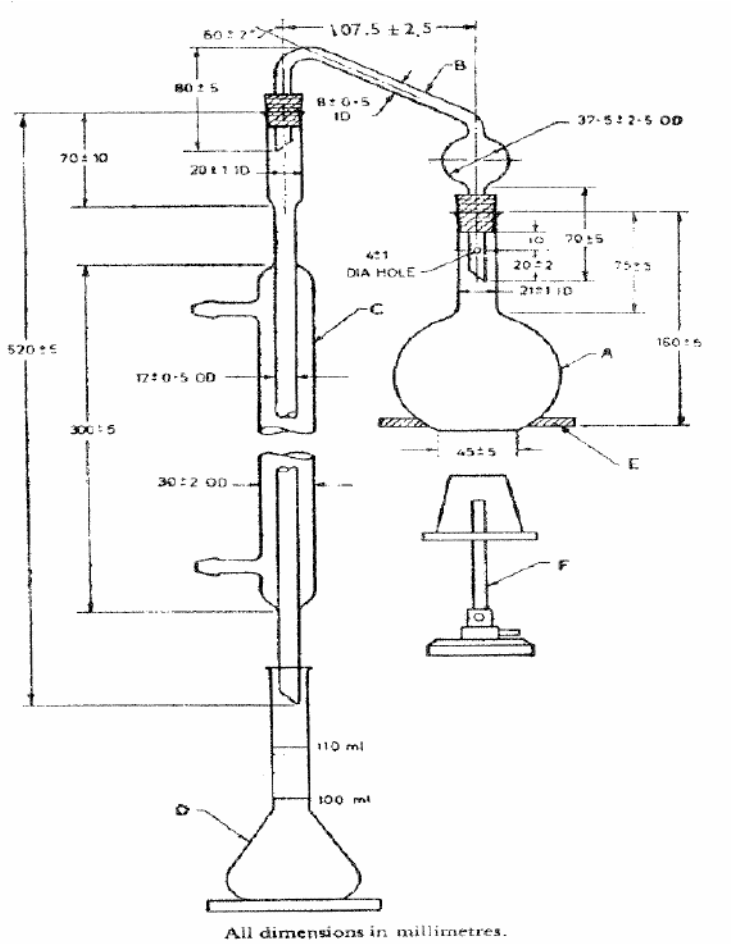
	distilled water and make up to 1000 mL. (iii). For standardisation of sodium thiosulphate, pipette 25 mL of this solution into a 250 mL conical flask. Add 5 mL of concentrated hydrochloric acid and 15 mL of a 10% potassium iodide solution. (iv). Allow to stand in dark for 5 min and titrate with sodium thiosulphate solution using starch as indicator. End point is change of blue color to green. $$N = \frac{25 \times W}{49.03 \times V}$$ Where, N = Normality of the sodium thiosulphate W = Weight in g of the potassium dichromate, and V = Volume in mL of sodium thiosulphate solution required for titration. 5. Potassium dichromate (dried at 105±2 °C for one hour).
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	Oil/fat may be weighed accurately following the Table given below: Expected Iodine Weight to be taken for value estimation (g)

Expected Iodine Value	Weight to be taken for estimation (g)	
	Maximum	Minimum
5	6.3460	5.0770
10	3.1730	2.5384
50	0.6612	0.5288
100	0.3173	0.2538
150	0.2125	0.1700
200	0.1586	0.1269

1. Weigh accurately an appropriate quantity of the dry oil/fat as indicated in the Table above, into a 500 mL glass stoppered conical flask, 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% of Wijs solution over that actually needed. Pipette 25 mL of Wijs solution and replace the glass stopper after wetting with potassium iodide solution.
3. Swirl for proper mixing and keep the flasks in dark for 30 min for non-drying and semi-drying oils and one hour for drying oils.
4. Carry out a blank simultaneously.
5. After standing, add 15 mL of potassium iodide solution, followed by 100 mL of recently boiled and cooled water, rinsing in the stopper also.
6. Titrate the liberated iodine with standardized sodium thiosulphate solution, using starch as indicator until the blue color formed

	disappears after thorough shaking with the stopper on. 7. Conduct blank determinations in the same manner as test sample but without oil/fat. 8. Slight variations in temperature appreciably affect titre of iodine solution as chloroform has a high coefficient of expansion. 9. It is thus necessary that blanks and determinations are made at the same time.
Calculation with units of expression	Iodine value = $= \frac{12.69 \times (B-S) \times N}{W}$ Where, B = volume in mL of standard sodium thiosulphate solution required for the blank. S = volume in mL of standard sodium thiosulphate solution required for the sample. N = normality of the standard sodium thiosulphate solution. W = weight in g of the sample. Units: g of iodine per 100 g oil
Reference	1. AOAC 17th edn, 2000, Official method 920. 159 – Iodine absorption number of oils and fats 2. ISI Handbook of Food Analysis (Part XIII) – 1984 page 76. 3. AOCS Official Method Cd 1b-87: Iodine value of fats and oils: Cyclohexane 4. AOCS Official Method Cd 1D-92: Iodine value of fats and oils: Cyclohexane Acetic acid method
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Reichert-Meissl and Polenske Value**		
Method No.	FSSAI 02.011:2021	**Revision No. & Date**	0.0
Scope	Butter is distinguished from other fats by the presence of glyceryl esters of relatively low molecular weight fatty acids, especially butyric but also caproic, capric, caprylic, lauric and myristic acids. These acids are wholly or partially steam volatile and water soluble. The Reichert-Meissl value reflects the amount of butyric and caproic acids present and Polenske value chiefly caprylic, capric and lauric acids, with some contribution from myristic and even palmitic acid. The Reichert-Meissl value is the number of mLs of 0.1N aqueous sodium hydroxide solution required to neutralize steam volatile water-soluble fatty acids distilled from 5 g of an oil/fat under the prescribed conditions. It is a measure of water-soluble steam volatile fatty acids chiefly butyric and caproic acids present in either an oil or fat.		
Caution	1. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 2. Sulphuric acid: Concentrated Sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If Sulphuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death.		
Principle	The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute Sulphuric acid. The volatile acids are immediately steam distilled. The soluble volatile acid in the distillate is filtered out and estimated by titration with standard sodium hydroxide solution. Importance -These determinations have been used principally for analysis of butter and margarines. Butter fat contains mainly butyric acid glycerides. Butyric acid is volatile and soluble in water. No other fat contains butyric acid glycerides, and therefore, the Reichert-Meissl value of the butter fat is higher than that for any other fat. Coconut oil and palm kernel oil contain appreciable quantities of caprylic, capric and lauric acid glycerides. These fatty acids are steam volatile but not soluble in water, and hence give high Polenske value.		
Apparatus / Instruments	1. General glass ware and apparatus (Refer 2.0 at page no. 1) 2. An all-glass distillation assembly conforming to specifications as per AOCS Methods Cd 5-40 or AOAC- 17th Edn.,2000 (925.41, Chapter 41 page 14) or distillation apparatus as shown in the diagram below:		

	 All dimensions in millimetres. 3. Beaker-25 mL 4. Graduated cylinder-100 mL 5. Pipette-100 mL 6. Graduated burette. 7. Asbestos board with a hole about 65 mm diameter for supporting the flask over the burner. During distillation the flask shall fit snugly into the hole of the board to prevent the flame from impinging on the surface of the flask above the hole. 8. Bunsen burner sufficiently large to allow completion of distillation in the prescribed time.
Materials and Reagents	1. Glycerol 2. Sodium hydroxide 3. Pumice stone grains 4. Sulphuric acid 5. Phenolphthalein indicator 6. Ethyl alcohol

Preparation of reagents	1. Concentrated sodium hydroxide solution: 50% (w/w) Dissolve Sodium Hydroxide in equal weight of water and store solution in a polypropylene bottle. Use clear solution free from deposit. 2. Dilute Sulphuric acid solution: Approximately 1.0N 3. Sodium hydroxide solution: 0.1N solution in water, accurately standardized 4. Phenolphthalein indicator: Dissolve 0.1 g of phenolphthalein in 100 mL of ethyl alcohol 5. Ethyl alcohol: 90% by volume and neutral to phenolphthalein.
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Weigh accurately 5 ± 0.1 g of filtered oil or fat sample into a clean, dry, 300 mL distilling flask. 2. Add 20 mL of glycerine and 2 mL of concentrated sodium hydroxide solution, and heat with swirling over a flame until completely saponified, as shown by the mixture becoming perfectly clear. 3. Cool the contents slightly and add 90 mL of boiling distilled water, which has been vigorously boiled for about 15 min After thorough mixing, the solution should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating over-heating), repeat the saponification with a fresh sample of the oil or fat. If the sample is old, the solution may sometimes be dark and not clear. 4. Add about 0.6 - 0.7 g of pumice stone grains, and 50 mL of dilute Sulphuric acid solution. Immediately connect the flask to the distillation apparatus. 5. Place the flask on asbestos board so that it fits snugly into the aperture. This will prevent the flame from impinging on the surface of the flask above the level of the liquid and avoid super heating. 6. Heat very gently until the liberated fatty acids melt and separate. 7. Then set the flame so that 110 mL of distillate shall be collected within 19 to 21 min. 8. The beginning of the distillation is to be taken as the moment when the first drop of the distillate falls from the condenser in the receiving flask. 9. Keep the water in the condenser flowing at a sufficient speed to maintain the temperature of the outgoing water from the condenser between 15 and 20 °C. 10. Collect the distillate in a graduated flask. 11. When the distillate exactly reaches the 110 mL mark on the flask, remove the flame and quickly replace the flask by a 25 mL measuring cylinder. 12. Stopper the graduated flask and without mixing place it in a water bath maintained at 15 °C for 10 min so that the 110 mL graduation mark is 1 cm below the water level in the bath. 13. Swirl round the contents of the flask from time to time. Remove the graduated flask from the cold water bath, dry the outside and mix

	the content gently by inverting the flask 4 to 5 times without shaking. Avoid wetting the stopper with the insoluble acids. 14. Filter the liquid through a dry, 9 cm Whatman No. 4 filter paper or equivalent. Reject the first 2-3 mL of the filtrate and collect the rest in a dry flask. 15. The filtrate should be clear. Pipette 100 mL of the filtrate and add 5 drops of the phenolphthalein solution and titrate against standard 0.1N sodium hydroxide solution. 16. Run a Blank Test without the fat but using the same quantities of the reagents. Polenske Value: 17. After titrating, the soluble volatile acids detach the still head and rinse the condenser with three successive 15 mL portions of cold distilled water passing each washing separately through the measuring cylinder, 110 mL graduated flask and the filter paper and allow all of it to pass through. Discard all the washings. 18. Place the funnel on a clean conical flask. Dissolve the insoluble fatty acids by three similar washings of the condenser, the measuring cylinder, the 110 mL flask with stopper, and the filter paper with 15 mL portions of ethyl alcohol. 19. Combine the alcoholic washings in a clean flask, add 5 drops of phenolphthalein indicator solution, and titrate with standard (0.1N) sodium hydroxide solution.
Calculation with units of expression	Reichert-Meissl Value$= (A - B) \times N \times 11$ where, A = Volume in mL of standard sodium hydroxide solution required for the test; B = Volume in mL in standard sodium hydroxide solution required for the blank; and N = Normality of standard sodium hydroxide solution. **Calculation of Polenske Value:** Polenske value$= 10 \times V \times N$ where, V = Volume in mL of standard sodium hydroxide solution required for the test; and N = Normality of the standard sodium hydroxide solution. Note: - Unless the directions are followed in every detail reproducible results cannot be obtained.
Reference	1. ISI Handbook of Food Analysis (Part XIII) – 1984 page 81) 2. AOAC 17th edn, 2000. Official method 925.41 Acids (volatile) in oils and fats.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Bellier Test (turbidity temperature) acetic acid method		
Method No.	FSSAI 02.012:2021	**Revision No. & Date**	0.0
Scope	Oils containing long chain saturated fatty acids give a precipitate at a particular temperature.		
Caution	1. Potassium hydroxide: It is corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation.		
Principle	It is specific for each oil. Oils give a precipitate, when their alcoholic soap solution is treated with dilute acetic acid solution and 70% ethyl alcohol.		
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Conical flask - 100 mL capacity with cork 3. Thermometer (0 – 60 °C calibrated to read 0.5 °C) 4. Water bath		
Materials and Reagents	1. Purified / Rectified spirit 2. Potassium hydroxide 3. Acetic acid 4. Phenolphthalein 5. Aluminium granules (or aluminium foil pieces)		
Preparation of reagents	1. Purified / Rectified spirit: Reflux 1.2 L of rectified spirit for 30 min in a distillation flask with 10 g of caustic potash and 6 g of granulated aluminium (or aluminium foil pieces). Distil and collect one liter after discarding the first 50 mL. Use this purified rectified spirit for preparation of all the reagents. 2. Alcohol 70% (by volume): Dilute 700 mL of alcohol to 950 mL with distilled water and check the strength by specific gravity determination and adjust if necessary. The specific gravity of 70% alcohol at 15.5 °C is 0.8898 and 30 °C is 0.8807. The final strength should be checked accurately. 3. Alcoholic potash (1.5N): Dissolve 8.5 g Potassium hydroxide in 100 mL purified rectified spirit. It is preferable to keep this solution in a dark color bottle. 4. Dilute acetic acid: Mix one volume of glacial acetic acid with two volumes of distilled water. 5. Phenolphthalein indicator: Dissolve 0.5 g of phenolphthalein in 50 mL of purified rectified spirit and mix the solution with 50 mL of distilled water.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Measure with the aid of a pipette one ml of the filtered sample of oil in a flat-bottom 100 mL conical flask (preferably with a long neck),		

	add 5 mL of 1.5N alcoholic potash and saponify completely by heating over a boiling water-bath using an air condenser (about 1.3 meters long) to avoid loss of alcohol as far as possible. Complete saponification usually takes about 10 min. During saponification, swirl the flask several times. 2. Cool, add 0.1 mL of phenolphthalein indicator, neutralise exactly by adding carefully dilute acetic acid and then add an extra amount of 0.4 mL (accurately measured). 3. Add 50 mL of 70% alcohol and mix. Fit a thermometer (0° to 60 °C reading to 0.5 °C, accurately calibrated) into the flask, with the aid of a velvet cork in such a way that the bulb of the thermometer is immersed in the liquid but does not touch the bottom of the flask. 4. Heat the flask gently over the water-bath until the temperature reaches 50 °C and the solution is clear. 5. Allow the flask to cool in air with frequent shaking until the temperature falls gradually to 40 °C (in case of pure groundnut oil turbidity appears at 39 to 41 °C). 6. Then, cool the flask with constant shaking by occasional immersion in a cooling bath maintained at 15 °C (±1°C) so that the temperature drops roughly at the rate of 2 °C per min. Note the temperature at which the first distinct turbidity appears is the turbidity temperature. 7. This turbidity temperature is confirmed by a little further cooling, which would result in deposition of the precipitate. 8. Dissolve the precipitate by gently heating the contents to 50 °C in a water-bath, again cool as described above and make a duplicate determination of the turbidity temperature. 9. The mean of the two values is taken as the true turbidity temperature. Duplicate shall agree within ±0.5°C.
Inference (Qualitative Analysis)	It is essential that stirring is continuous and moderate while the contents are being cooled in the cooling bath. Violent shaking or agitation would be avoided as it will affect the result adversely.
Reference	ISI Handbook of Food Analysis (Part XIII) 1984 - page 90
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of Sesame Oil (Baudouin test)		
Method No.	FSSAI 02.013:2021	**Revision No. & Date**	0.0
Scope	Sesamolin is a lignan, present in sesame oil.		
Caution	Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases.		
Principle	The development of pink color with furfural solution in the presence of hydrochloric acid indicates the presence of sesame oil. The color is produced on account of reaction of furfural with sesamolin present in sesame oil.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Glass stopper test tubes / measuring cylinders.		
Materials and Reagents	1. Hydrochloric acid (concentrated) Sp. Gr. 1.19 2. Furfural solution		
Preparation of reagents	Furfural solution (2 percent furfural–freshly distilled in ethyl alcohol)		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Take 5 mL of the oil or melted fat in a 25 mL measuring cylinder (or test tube) provided with a glass stopper and add 5 mL of concentrated hydrochloric acid and 0.4 mL of furfural solution. Insert the glass stopper and shake vigorously for two min. 2. Let it stand and allow the mixture to separate. 3. The development of a pink or red color in the lower acid layer indicates presence of sesame oil. 4. Confirm by adding 5 mL of water and shaking again. 5. If the color in acid layer persists, sesame oil is present and if the color disappears it is absent. (As furfural gives violet tint with hydrochloric acid, it is necessary to use the dilute solution specified) **Note:** Test the sample for the presence of coloring matter that are chromogenic in presence of Hydrochloric acid. For this purpose, take 5 mL of the sample in a 25mL measuring cylinder provided with a glass stopper and shake with 5mL of concentrated hydrochloric acid. If there is no development of pink or red color in the aqueous layer apply the test as above. If pink or red color develops in the aqueous layer, remove the red acid layer which collects at the bottom and repeat the procedure until no further coloration takes place. After complete removal of Hydrochloric acid layer perform the test as prescribed above.		
Inference (Qualitative Analysis)	The development of a pink or red color in the lower acid layer indicates presence of sesame oil, provided no other interfering substances are present.		
Reference	1. ISI Handbook of Food Analysis (Part XIII)-1984 Page 86 2. AOAC 17th edn,2000, Official method 893.01-Oil (sesame) in Oils		

	and Fats Modified Villavecchia Test 3. AOCS, 6th edn, 2012. Official Method Cb2-40 4. Codex Alimentarius – Recommended Method 25, 1970
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of presence of Cottonseed Oil (Halphen's test)		
Method No.	FSSAI 02.014:2021	**Revision No. & Date**	0.0
Scope	Cotton seed oil contains Cyclopropanoid fatty acids.		
Caution	1. Sulphur: Exposure to sulphur vapours may be irritating to the eyes. Dry - eye contact with dusts may be irritating to the throat and lungs, Ingestion of dry sulphur may cause irritation of the mouth and sore throat. 2. Carbon disulphide: Highly Flammable liquid and vapor [Danger Flammable liquids]; Causes skin irritation [Warning Skin corrosion/irritation]; Causes serious eye irritation [Warning Serious eye damage/eye irritation]; Suspected of damaging fertility; Suspected of damaging the unborn child [Warning Reproductive toxicity]; Causes damage to organs through prolonged or repeated exposure.		
Principle	The development of red color on heating the oil with a solution of sulphur in carbon disulphide indicates the presence of cottonseed oil. The test is also given by Hempseed oil, Kapokseed oil / oils and fats containing cyclopropenoid fatty acids (such as sterculic and malvalic acid). Hydrogenation and deodorization wholly or partially destroy the chromogens and react with diminished intensity. A positive reaction is not given by oil heated to 250 °C or above. The fat of animals fed on cottonseed meal (butter, lard) or other cottonseed products may give faint positive reaction by this test.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Test tubes 3. Water bath 4. Oil bath or Brine bath maintained at 110 – 115 °C		
Materials and Reagents	1. Sulphur 2. Carbon disulphide 3. Amyl alcohol 4. Oil 5. Sodium chloride		
Preparation of reagents	1. Sulphur solution: Prepare a 1% (w/v) solution of sulphur in carbon disulphide and then add an equal volume of amyl alcohol.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Take about 5 mL of the oil or melted fat in a test tube and add to it an equal volume of the sulphur solution. 2. Mix thoroughly by shaking and heat gently on a water bath (70 – 80 °C) for a few min with occasional shaking until the carbon disulphide has boiled off and the sample stops foaming. 3. Place the tube in an oil bath or a saturated brine-bath maintained at 110-115 °C and hold for 2.5 h. 4. A red color at the end of this period indicates the presence of		

	cottonseed oil. 5. The test is sensitive to the extent of 0.5% cottonseed oil in other oils.
Inference (Qualitative Analysis)	A red color at the end of the method of analysis indicates the presence of cottonseed oil in oils and fats.
Reference	1. ISI Handbook of Food Analysis of (Part XIII)-1984 Page 86 2. AOAC 17th edn, 2000, Official method 197.02-oil (cottonseed) in oils and fats 3. FAO Manuals of Food Quality Control 14 / 8 Page 271 4. AOCS, 6th edn, 2012. Official Method Cb 1-25 5. CODEX Alimentarious Commission – Recommended Method (RM)- 23, 1970
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Cloud Point in Palmolein (and test for presence of palmolein in other oils)		
Method No.	FSSAI 02.015:2021	**Revision No. & Date**	0.0
Scope	Palmolein has a 'cloud point' of approximately 10 °C. So, whenever the temperature drops to 10 °C, the palmolein molecules crystallise; making the oil appears cloudy. At lower temperatures, the cloudy oil becomes solid.		
Principle	The cloud point is that temperature at which (under the conditions of this test) a cloud is induced in the sample caused by the first stage of crystallization.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Oil sample bottle, 115 mL (4 oz) 3. Thermometer, range 2 – 68 °C 4. Water bath made up of water, chipped ice and water or chipped ice, salt and water, depending upon the temperature required. The temperature of the water bath shall not be less than 2 °C and not more than 5 °C of the cloud point.		
Materials and Reagents	Oils / Fats		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. The sample must be completely dry before conducting the test. Heat 60 - 75 g of sample to 130 °C just before the test. 2. Pour ca 45 mL of the heated fat into an oil sample bottle. Place the bottle in a water bath. 3. Begin to cool the bottle in the water bath, stirring enough using the thermometer to keep the temperature uniform. 4. When the sample has reached a temperature ca 10 °C above the cloud point, begin stirring steadily and rapidly in a circular motion so as to prevent super-cooling and solidification of fat crystals on the sides or bottom of the bottle. 5. From this point on, do not remove the thermometer from the sample, since doing so may introduce air bubbles which will interfere with the test. 6. Maintain the test bottle in such a position that the upper levels of the sample in the bottle and the water in the bath are about the same. 7. Remove the bottle from the bath and read the temperature. The bottle should be inspected regularly. 8. The cloud point is that temperature at which that portion of the thermometer immersed in the oil is no longer visible when viewed horizontally through the bottle.		
Inference (Qualitative Analysis)	This test is useful for the detection of palmolein in groundnut oil. Presence of palmolein over 10 percent in groundnut oil readily gives cloud at a higher temperature than that of groundnut oil due to the presence of palmitic glycerides in higher amounts in palmolein / palm oil.		
Reference	1. AOCS, 6th edn, 2012. Official Method Cc 6-25 2. Manual methods of Analysis for Adulterants and Contaminants in Foods , ICMR (1990) Page 4		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Test for presence of Rice Bran Oil		
Method No.	FSSAI 02.016:2021	**Revision No. & Date**	0.0
Scope	Rice bran oil is the oil extracted from the hard outer brown layer of rice called chaff (rice husk). A component of rice bran oil is the oryzanol (major compound-antioxidant γ-oryzanol) at around 2% of crude oil content.		
Caution	1. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 2. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 3. Benzene: Benzene has long been recognized as capable of increasing the risk of leukemia and other blood disorders, and benzene can damage blood-forming cells in the bone marrow. 4. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses.		
Principle	Oryzanol in rice-bran oil is isolated using 30% aqueous Potassium hydroxide solution and detected on thin-layer Chromatographic plate.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Conical flasks, 250 mL capacity - 4 Nos., 100 mL capacity - 2 Nos. 3. Thin layer Chromatographic plates (0.25 mm) prepared by coating slurry of silica gel G. on glass plate of 20 x 10 cm dimension. 4. Iodine chamber for visualization of spots. 5. Spotting capillaries 6. Separating funnel (100 mL capacity) 7. Hot water bath		
Materials and Reagents	1. Potassium hydroxide 2. Hydrochloric acid 3. Blue litmus paper 4. Diethyl ether AR grade 5. Sodium sulphate anhydrous 6. Benzene 7. Acetic acid 8. Chloroform AR grade		

Preparation of reagents	1. Aqueous Potassium hydroxide solution 30% 2. Hydrochloric acid dilute 3. Benzene - acetic acid (100: 1 v/v)
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Take 20 mL of the oil in a 100 mL capacity separating funnel and add to it equal volume of aqueous Potassium hydroxide solution. 2. Shake the contents gently but constantly for 10 min. 3. Keep the separating funnel on a stand for about 45 min to allow the separation of alkali layer. 4. Draw the alkali layer and neutralize with dilute hydrochloric acid solution. Confirm the neutralization with blue litmus paper. 5. Extract this salt solution with diethyl ether (20 mL x 3 times). 6. Wash the diethyl ether extract with distilled water and dry on anhydrous sodium sulphate. 7. Evaporate the solvent on hot water bath to obtain residue. 8. Spot the residue in chloroform on TLC/HPTLC plate. 9. Develop the TLC/HPTLC plate in pure benzene: acetic acid mixture. Allow the solvent front to move a distance of 15 cm. 10. Visualize the spots in iodine chamber. 11. Appearance of a spot between R_f 0.7 to 0.75 indicates the presence of rice bran oil. 12. Run a control by taking a sample of rice bran oil and compare the spot given by test sample under identical conditions.
Inference (Qualitative Analysis)	The above method can detect rice-bran oil in other edible vegetable oils up to the minimum of 5% level.
Reference	Manual methods of Analysis for Adulterants and Contaminates in Foods ICMR (1990) Page 5
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of Linseed Oil (Hexabromide test)		
Method No.	FSSAI 02.017:2021	**Revision No. & Date**	0.0
Scope	Linseed oil, also known as flaxseed oil or flax oil, is a colorless to yellowish oil obtained from the dried, ripened seeds of the flax plant (*Linum usitatissimum*). It contains linolenic acid, which gives hexa bromide on bromination.		
Caution	1. Liquid bromine: Breathing bromine gas could causes cough, trouble breathing, gets a headache, causes irritation of mucous membranes (inside mouth, nose, etc.), dizzy, watery eyes. Getting bromine liquid or gas on skin could cause skin irritation and burns. The use of safe and suitable pipette i.e. Lunge-Ray pipette, is suggested for the handling and addition of bromine. 2. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 3. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity.		
Principle	The formation of a precipitate of hexabromide when the oil in chloroform is treated with bromine, followed by alcohol and ether in cold condition indicates the presence of linseed oil.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no.1) 2. Boiling tubes 3. Ice water bath		
Materials and Reagents	1. Chloroform – A.R 2. Liquid bromine – A.R 3. Ethyl alcohol 4. Diethyl ether		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Pipette one mL of the oil into a boiling tube (wide-mouthed 100 mL capacity). 2. Add 5 mL of chloroform and about one mL of bromine drop-wise till the mixture becomes deep red in color and cool the test-tube in an ice water-bath. 3. Add about 1.5 mL of rectified spirit drop-wise while shaking the mixture until the precipitate forms, which was first formed just dissolves. 4. Add 10 mL of diethyl ether. Mix the contents and place the tube within the ice water-bath for 20 min. Appearance of precipitate indicates the presence of linseed oil.		

Inference (Qualitative Analysis)	1. This test is not applicable for detecting linseed oil in Mahua oil. 2. The test is also given by fish oils and fats containing highly unsaturated fatty acids. It has been observed that in low erucic rapeseed oil, and Mahua oil having linolenic acid content greater than 12.0% may also give positive test. The results obtained in such cases have to be viewed with caution. Experiments conducted with these oils with or without added linseed oil have shown that, if linseed oil is present even at 1% level, hexabromide insoluble in cold ether are formed within 20 min. Any hexabromides insoluble in cold ether formed after 20 min need not be taken for the presence of linseed oil. 3. An explanation for this behavior of these oils can be given on the basis of glyceride structure. Oils and fats are mixed triacylglycerides i.e. different fatty acid are present in each of the positions of the glycerol molecule. Exception to this rule is the oil /fat containing a particular fatty acid in amounts greater than 50 % where such a fatty acid may take all the three positions of the glycerol molecule giving rise to simple triacylglycerides. Linseed oil is such an example containing greater than 50% Linolenic acid.
Reference	1. Manual of Methods of Analysis for Adulterants and Contaminants in Foods, ICMR (1990) Page 5 2. ISI Handbook of Food Analysis Part (XIII) – 1984 page 86
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Polybromide test for Mustard Oil		
Method No.	FSSAI 02.018:2021	**Revision No. & Date**	0.0
Scope	This test for the presence of fatty acids with more than two non conjugated double bonds is more reliable on fatty acids than on glycerides in which one of the three fatty acids in combination may be polyunsaturated.		
Caution	1. Liquid bromine: Breathing bromine gas could causes cough, trouble breathing, gets a headache, causes irritation of mucous membranes (inside mouth, nose, etc.), dizzy, watery eyes. Getting bromine liquid or gas on skin could cause skin irritation and burns. The use of safe and suitable pipette i.e. Lunge-Ray pipette, is suggested for the handling and addition of bromine. 2. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity.		
Principle	An ethereal solution of the fat or fatty acid is treated with bromine. The formation of a precipitate gives a qualitative indication of the presence of fatty acids with three or more non conjugated double bonds.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Conical Flask 100 mL capacity 3. Burette with a finely drawn out jet		
Materials and Reagents	1. Diethyl ether 2. Bromine		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Dissolve approximately 3 g of clear fat in 25 mL diethyl ether in the conical flask. 2. Place the flask in a melting ice bath for 15 min and then slowly add 1 mL bromine drop wise from burette with continuous swirling and cooling (the first half mL in 20 min and the remainder in 10 min). 3. Cool the flask and keep it in the ice bath for a further 3 h. If a precipitate forms, the reaction is considered positive.		
Inference (Qualitative Analysis)	Precipitate indicates the presence of fatty acids with three or more non conjugated double bonds.		
Reference	Laboratory Handbook for Oil and Fat Analysis, Cocks and Reid, page 147- 148		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of fatty acid composition of oils and fats by gas liquid chromatography		
Method No.	FSSAI 02.019:2021	**Revision No. & Date**	0.0
Scope	Triacylglycerols are the predominant components of most food fats and oils. A triacylglycerol is composed of glycerol and three fatty acids.Fatty acids composition differs for each of edible oils.		
Caution	1. Boron trifluoride: Boron trifluoride is extremely corrosive. Acute inhalation exposure of boron trifluoride may result in sneezing, hoarseness, choking, laryngitis, and respiratory tract irritation. 2. Sodium Carbonate: Eye contact can cause permanent corneal injury and possible burns. Avoid ingestion or inhalation of dust. Due to these potential hazards, sodium carbonate should be handled with care. 3. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 4. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	The methyl esters of fatty acids are formed using boron trifluoride or methanol and alkali and separated by gas – liquid chromatography using a flame ionization detector. The elution pattern of methyl esters can be compared with authentic oils for identification. Alternate method Methyl esters can also be prepared without the use of boron trifluoride. This involves methyl esterification of the fatty acids in an alkaline medium and is suitable for neutral oils and fats with an acid value less than 2.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1). 2. Gas liquid chromatograph with the following characteristics: a) Injection system heated to a temperature of 20 – 50 °C higher than the column. b) Oven – capable of heating the column to at least 220 °C and maintaining the temperature to within ±1 °C. If temperature programming is to be employed, twin columns are recommended. c) Packed column - may be glass or stainless steel. However, glass is preferred as steel may decompose polyunsaturated fatty acids having more than 3 double bonds. Some successful column packing with column length, internal diameter and operating temperature are as follows i) 12- 15% ethylene glycol succinate on 100 / 120 mesh gas chrom P (2m x 4 mm, at 180 °C) ii) 2- 10 % Apizon –L on 80/ 100 mesh Chromosorb W or Celite (2 m x 4 mm at 220 °C)		

	iii) 10 % Butan-1-4 diol succinate on 80 / 100 mesh Chromosorb W or celite (2 m x 4 mm at 175 °C) iv) 3 % SE – 30 on 100 / 120 mesh Chromosorb –G silanised (2m x 3mm at190 °C) Alternatively, capillary column HP88, SP2560 column can be utilized. Condition the newly prepared column by disconnecting the detector and heating the column in the oven to the normal operating temperature for 16 h while running the carrier gas at a rate of 20 – 60 mL/min v) Detector – Flame ionization detector – capable of being heated to a temperature above that of the column 3. Syringe –10 µL graduated in 1/10th of a microlitre 4. Recorder – electronic with high precision with rate of response below 1.5 second, width of paper 25cm, paper speed 25-150 cm/hours 5. Integrator or calculator for rapid and accurate calculations. 6. 50 and 100 mL boiling flasks 7. Reflux condenser 8. Graduated pipette – 10 mL 9. Test tubes with ground stoppers 10. 250 mL Separating funnels
Materials and Reagents	1. Carrier Gas – Inert gas (nitrogen, helium, argon) thoroughly dried and containing less than 10 mg / kg of oxygen 2. Auxiliary gas Hydrogen 99.9% minimum purity. Free from organic impurities, air or oxygen 3. Reference standards – a mixture of methyl esters of fatty acids (FAMEs) or methyl esters of oils of known purity preferably similar to the fatty matter being analyzed (CRM 47885) 4. Methanol 5. Sodium hydroxide 6. Sodium Carbonate 7. Boron trifluoride 8. Heptane- Chromatographic quality 9. Redistilled petroleum Ether 40 – 60 °C 10. Anhydrous Sodium sulphate. 11. Sodium chloride. 12. Methyl red 13. Fatty acid methyl esters(FAMEs) of C_{13} and C_{11} (Internal standards) Additional chemicals for Alternate Method 14. Potassium hydroxide 15. Nitrogen, containing not more than 0.5 mg/Kg of oxygen
Preparation of reagents	1. Methanolic Sodium hydroxide solution - approx 0.5 N. Dissolve 2 g of Sodium Hydroxide in 100 mL methanol containing not more than 0.5% m/m water. When the solution has to be stored for considerable time, a small amount of white precipitate of Sodium Carbonate may be formed. This has no effect on the preparation of the methyl esters 2. Methanolic solution of Boron trifluoride – 12 - 15% m/m, 14 and 50% solutions are commercially available. The methanolic solution of boron trifluoride should be stored in a refrigerator

	3. Saturated solution of Sodium chloride. 4. Methyl red – 1 g / L in 60% alcohol Additional reagents for alternate method 5. Methanol containing not more than 0.5% water 6. Methanolic Potassium hydroxide solution – approx 1 N. Dissolve 5.6 g Potassium hydroxide in 100 mL of methanol containing not more than 0.5% m/m water (anhydrous methanol)
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Prepare the methyl esters of the fatty acids. The method using boron trifluoride gives good results and is preferable to alternative methods which may be used when boron trifluoride is not available. Because of the toxic character of boron trifluoride various operations must be performed under a ventilated hood. 2. All glass ware must be washed with water immediately after use. If the oil or fatty acids include fatty acids containing more than 2 double bonds it is advisable to purge the air from the methanol and the flask by passing a stream of nitrogen into the methanol for a few min. 3. Transfer about 350 mg of clear oil to a 50 mL conical flask, and add 6 mL of 0.5 N methanolic sodium hydroxide solution, 7 mL of boron trifluoride solution and a boiling chip. 4. Fit the condenser to the flask. Boil under reflux until the droplets of oil disappear (5- 10 min). 5. Add the appropriate amount of boron trifluoride solution with a bulb or automatic pipette through the top of the condenser. Continue boiling for 2 min. 6. Add 2- 5 mL of heptane to the boiling mixture through the top of the condenser. Continue boiling for 1 min. 7. Withdraw the source of heat and then remove the condenser. 8. Add a small amount of saturated Sodium Chloride solution to the flask in order to bring the level of liquid into the neck of the flask. 9. Transfer about 1 mL of the upper layer (Heptane solution) into a test tube with a ground glass neck and add a little anhydrous Sodium Sulphate to remove any trace of water. 10. This solution will contain about 5 – 10% of methyl esters and may be injected directly into the column of gas liquid chromatograph. Alternate method for preparation methyl esters 1. If the oil includes fatty acids containing more than 2 double bonds, it is advisable to purge the air from the methanol and the flask by passing a stream of nitrogen into the methanol for a few min. 2. Transfer about 4 g of clear sample oil into a 100 mL round bottomed or conical flask. 3. Add about 40 mL of methanol, 0.5 mL of methanolic Potassium hydroxide solution and a boiling chip. 4. Fit under a reflux condenser, stir and bring to boil. The solution should become clear (5-10 min). 5. Cool under running water and transfer the contents to a 125 mL

	separating funnel, rinsing the flask with 20 mL of heptane. 6. Add about 40 mL water, shake and allow to separate. The esters pass into the upper heptane layer. 7. Separate. Extract the aqueous layer again with 20 mL heptane. 8. Combine the two extracts and wash them with several 20 mL portions of water. Separate and dry the ester solution over anhydrous Sodium sulphate. 9. Filter through cotton wool into a 50 mL conical flask and evaporate solution to approx 20 mL on a water bath while passing a stream of nitrogen. 10. Add known quantity of Internal standard FAME. **Column Chromatography** Programme GC to maintain column temperature of 185 °C and detector temperature at 200 °C. Inject 0.1 – 2 µL of 5- 10% of heptane solution of methyl esters by piercing the septum of the inlet port. Withdraw needle and note formation of a small peak on the chart paper due to solvent making start reference point. It is possible to work with lower column temperature where the determination of acids below C_{12} is required or higher temperature when determining fatty acids above C_{20}. It is also possible to employ temperature programming to take care of both situations. Analyse reference standard mixture of known composition in the same operating conditions as those employed for the sample and measure the retention distances or retention times for the common fatty esters. Identify the peaks for the sample from the graph. If an integrator is used obtain the figures from it. Fatty acids appear on the chart in increasing number of carbon atoms and increasing unsaturation. Thus C_{16} appears before C_{18}, $C_{18:1}$ before $C_{18:2}$ and so on. Quantification. Known quantities of each of the FAMEs (reference standards) mixed along with known quantity of FAME of internal standard (C_{13}or C_{11}) in heptanes (~5-10% of FAME in heptane). This solution is subjected to GC analysis as per the above conditions. Peak areas are noted.
Calculation with units of expression	1. Determination of response factor of each of the FAME. Response factor of each FAME (K_n) [n=Carbon with double bond e.g., 18, 18:1, 18:2 etc] $$K_n = \frac{A_E \times m_R}{A_R \times m_E}$$ A_R = Peak area - Reference FAME standard- n

	A_E = Peak area - internal FAME standard m_R = Mass (mg) - Reference FAME standard -n (Known) m_E = Mass (mg) - internal FAME standard (Known) 2. Determination of each FAME in the mixture (methylated fat/oil) $\text{Percentage of the each FAME}_n = \frac{100 \times A_{Rn} \times m_E \times K_n}{A_E \times m}$ A_{Rn} = Peak area FAME $_n$ in the mixture. m = mass (mg) – methylated fat / oil.
Reference	1. IUPAC 2.301, 2.302 (1979)/FAO Manuals of Food quality Control 14/8, pages 274 – 281. 2. AOAC 17th edn, 2000 Official method 969.33 and 969.22 Fatty acids in oils and fats Preparation of methyl esters/Gas chromatographic method. Fatty Acid Composition by capillary GLC. 3. AOCS, 6th edn, 2012. Official Method Ce 1a-13
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of animal body fat in vegetable fat (Microscopic examination of fat crystals)		
Method No.	FSSAI 02.020:2021	**Revision No. & Date**	0.0
Scope	Animal body fats such as beef tallow and lard have been shown to contain trisaturated glycerides.		
Caution	1. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 2. Glycerol: Glycerol when taken orally may cause headache, nausea and vomiting, and less frequently, diarrhoea, thirst, dizziness and mental confusion. Cardiac arrhythmias have been reported. Glycerine may cause severe dehydration in previously dehydrated patients.		
Principle	Microscopic examination of fat crystals - On crystallization these glycerides exhibit a characteristic crystalline appearance when viewed under microscope. The procedure recommended by Williams Sutton for the microscopy of fat crystals have been suitably modified and given.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Ice bath 3. Microscope 4. Slides		
Materials and Reagents	1. Fat 2. Diethyl ether 3. Ethyl alcohol 4. Glycerole		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Take about 2 g of melted fat samples in test tubes and mix with 10 mL diethyl ether. 2. Plug the tubes with cotton and allow to stand for 30 min in ice water for 24 h at 20 °C (slow crystallization gives bigger crystals). 3. In certain cases, it is preferable to first crystallize with a stronger solution of fat from a mixture of ether and ethyl alcohol (1:1). 4. In such cases separate the crystals by filtration and recrystallise in ether. 5. Place the crystals on a drop of glycerol previously taken on a microscopic slide. 6. Cover the crystals immediately with cover glass. 7. Examine the crystals under x 160 and finally x 400 magnifications. 8. The typical appearance of beef tallow crystallized into characteristic fan like tufts, the ends of which are more or less pointed can be seen. 9. Lard crystals are of chisel shaped. 10. Hydrogenated fats deposit smaller size crystals.		
Inference (Qualitative Analysis)	The size and shape of the crystals depend upon the strength of solution, amount of fat taken and the time allowed for crystallization		

Reference	Manual Methods of Analysis for Adulterants and Contaminants in Foods ICMR (1990) page 6
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method for Separation of Cholesterol by Reversed Phase Thin Layer Chromatography		
Method No.	FSSAI 02.021:2021	**Revision No. & Date**	0.0
Scope	Animal fats contain cholesterol, whereas vegetable fats contain sitosterol		
Caution	1. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 2. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 3. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 4. Sulphuric acid: Concentrated Sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If Sulphuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death.		
Principle	A preliminary separation of total sterols from the unsaponifiable matter is achieved on silica gel-G thin layer chromatography. Subsequently the sterols are separated by reversed phase chromatography on Kieselghur-G using liquid paraffin as stationary phase and aqueous acetone saturated with liquid paraffin as the mobile phase.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. TLC Chambers 3. TLC plates 4. Sprayer 5. Oven		
Materials and Reagents	1. Oils and Fats 2. Diethyl ether 3. Chloroform 4. Petroleum ether 5. Acetone 6. Water 7. Paraffin 8. Silicagel G 9. Iodine		

Preparation of reagents	p- Anisaldehyde reagent (1.5 g. p-anisaldehyde and 1.5 mL concentrated sulphuric acid in 27 mL ethyl alcohol)
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Separation of total sterols from unsaponifiable matter - Extract the unsaponifiable matter from the fat sample as per the method described elsewhere. Evaporate the ether and dissolve the residue in 5 mL of chloroform. **Thin Layer Chromatography** 2. Prepare 20 × 20 cm glass plates coated with 0.5 mm thick silica Gel-G. Air-dry the plates and activate at 110 °C for 2 h. 3. Cool the plates to room temperature spot the unsaponifiable matter along with the standard cholesterol on the plate. 4. Develop the plates in diethyl ether: petroleum ether (1: 1) solvent system. 5. Remove the plates when the solvent front reaches 14 cm height (it takes about 30 min). 6. Air-dry the plates and expose to iodine vapors for a while. Total sterols spot corresponding to standard spot of cholesterol appear as brown color spots. 7. Mark spots and scrape off with stainless steel blade into a test tube. 8. Extract the sterols using chloroform from silica gel. Separate the sterols by reversed phase thin-layer chromatography. Preparation of equilibrated aqueous acetone with liquid paraffin: 9. Take 300 mL of 4: 1 acetone: water in a separating funnel and add 30 mL of liquid paraffin (heavy grade). Shake well and keep for 18 h at room temperature for equilibration. 10. Separate the lower layer of liquid paraffin and dilute to 5% proportion with petroleum ether. 11. Use this for treating Kieselghur-G coated thin-layer chromatographic plates. 12. The upper acetone-water mixture serves solvent system to develop the paraffin treated plates. Preparation of the plates for reversed phase TLC/HPTLC: 13. Coat 20 × 20 cm glass plates of 0.5 mm thick layers with Kieseghur-G and water (1:2) slurry. 14. Air-dry the plates and then activate at 110 °C for an hour. 15. Cool the plates to room temperature in a desiccator. 16. For treatment of TLC/HPTLC plates with liquid paraffin, carefully dip the plate holding horizontally for a few seconds in a tray containing 5% liquid paraffin solution in petroleum ether as described above. Air-dry the plates. 17. Spot the sterols in chloroform isolated from unsaponifiable matter by a preliminary separation on silica gel-G thin layer chromatography on paraffin treated plates along with standard cholesterol. 18. Develop the plate using the solvent system of acetone: water (4:1) which was earlier equilibrated with paraffin.

	19. After the solvent front has ascended to a height of 15 cm remove the plate and air-dry. 20. Spray with p- Anisaldehyde reagent(1.5 g p-anisaldehyde and 1.5 mL concentrated sulphuric acid in 27 mL ethyl alcohol) followed by heating at 110 °C for 5 min. 21. The sterol spots appear as blue spots on pale pink background. Cholesterol appears at R_f 0.48 distinctly separated from other closely related sterols.
Inference (Qualitative Analysis)	The sterol spots appear as blue spots on pale pink background. Cholesterol appears distinctly separated from other sterols.
Reference	Manual Methods of Analysis of Adulterants and Contaminants in Foods, ICMR (1990) Page 7
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of animal body fat in vegetable fat based on the presence of unusual fatty acids in animal fats by gas liquid chromatography		
Method No.	FSSAI 02.022:2021	**Revision No. & Date**	0.0
Scope	The fatty acid composition of animal fat (beef tallow) and vanaspati containing animal fat show the presence of odd chain fatty acids and branched chain fatty acids namely $C_{15:0}$, $C_{15:1}$, $C_{17:0}$ and $C_{17:1}$. These fatty acids are absent in vegetable fats. On the basis of this fact it is possible to detect the presence of animal fat (beef tallow) in vegetable fats.		
Caution	1. Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 2. Sodium: Reacts violently with water, liberating highly flammable hydrogen gas; causes severe burns on eye or skin contact. Sodium reacts with the moisture on skin and other tissues to form highly corrosive sodium hydroxide. Contact of metallic sodium with the skin, eyes, or mucous membranes causes severe burns; thermal burns may also occur due to ignition of the metal and liberated hydrogen. 3. Methanol: Flammable liquid and vapor. May be fatal or cause blindness if swallowed. Cannot be made nonpoisonous. Harmful if inhaled or absorbed through skin. Causes irritation to skin, eyes, and respiratory tract. High vapor concentrations may cause drowsiness. May cause harm to the unborn child. Prolonged exposure may cause chronic effects. 4. Benzene: Benzene has long been recognized as capable of increasing the risk of leukemia and other blood disorders, and benzene can damage blood-forming cells in the bone marrow. 5. Dichloromethane: Higher levels of dichloromethane inhalation can lead to headache, mental confusion, nausea, vomiting, dizziness and fatigue. Skin Exposure - Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns.		
Principle	The methyl esters of fatty acids are formed using methanol and alkali and separated by gas – liquid chromatography using a flame ionization detector. The elution pattern of methyl esters can be compared with authentic oils for identification.		
Apparatus / Instruments	Refer Method No. - FSSAI 02.019:2021		
Materials and Reagents	1. Sodium 2. Anhydrous methanol 3. Acetic acid 4. Benzene		

	5. Dichloromethane
Preparation of reagents	Sodium methoxide: One g sodium dissolved in 100 mL of anhydrous methanol.
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	Preparation of fatty acid methyl esters - Take 30 to 50 mg of melted fat (1 drop) in a glass-stoppered test tube. Add 1 mL of dichloromethane/ benzene followed by 2 mL of 1% sodium methoxide solution. Hold the test tube at 60 °C for 10 min. Cool and add 0.1 mL of glacial acetic acid followed by 5 mL of distilled water and 5 mL petroleum ether (40-60 °C). Mix the contents. Allow the layers to separate. Take out about 2 mL of the upper layer containing the methyl esters in a small tube and concentrate it by passing nitrogen gas before injecting to gas chromatograph.
	Column Chromatography Gas chromatography - The instrument fitted with flame ionisation detector and stainless-steel column of 10 ft packed with 15% diethylene glycol succinate on C W (80-100 mesh), or any other intermediate polar stationary phase column. Alternative capillary column HP88 and SP2560 could be used. Maintain the column temperature at 185 °C, flow rate of carrier gas nitrogen at 2.8 kg/cm^2 (25 mL/min) and chart speed at 1 cm/min. Inject the methyl ester of fatty acids into injection port. Withdraw needle and note formation of a small peak on the chart paper due to solvent making start reference point. It is possible to work with lower column temperature where the determination of acids below C_{12} is required or higher temperature when determining fatty acids above C_{20}. It is also possible to employ temperature programming to take care of both situations. Analyse reference standard mixture of known composition in the same operating conditions as those employed for the sample and measure the retention distances or retention times for the common fatty esters. Identify the peaks for the sample from the graph. If an integrator is used obtain the figures from it.
Inference (Qualitative Analysis)	Fatty acids appear on the chart in increasing number of carbon atoms and increasing unsaturation.
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Foods, ICMR (1990) Page 8
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for Refined Winterized Salad Oils – Cold Test		
Method No.	FSSAI 02.023:2021	**Revision No. & Date**	0.0
Scope	Salad oil is any edible oil used in salad dressings. Salad oil is another term for a light tasting vegetable oil.		
Principle	This method measures the resistance of the test sample to crystallization and is commonly used as an index of the winterization and stearin removal process. Importance: The major characteristic is passing the Cold Test (5.5h minimum). Oil is often combined with other substances to achieve desired flavour and consistency.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Oil Sample bottle -115 mL (4 oz) must be clean, dry and free from dust particle 3. Bucket 4. Water bath – maintained at 25 °C		
Materials and Reagents	1. Paraffin 2. Ice 3. Water		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Filter around 200-300 mL of test sample through a filter paper and then heat the filtered portion of the test sample while stirring continuously till the temperature reaches 130 °C. 2. Fill an oil sample bottle completely full with the test sample, cork tightly and seal with paraffin. 3. Completely submerge bottle in bucket containing finely cracked ice and add water until it rises to top of the bottle. 4. Keep bucket filled solidly with ice by removing any excess water and adding ice when necessary. 5. After 5.5 h remove bottle and examine oil. If it is properly winterized, sample will be brilliant, clear and limpid.		
Inference (Qualitative Analysis)	Winterized salad oils will be brilliant, clear and limpid, even after cooling for 5-6 h as mentioned in the method.		
Reference	AOAC 17th edn, Official method 929.08 Salad oils (refined, winterized). Cold Test, AOCS, 6thedn, 2012. Official Method Cc 11-53		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Test for presence of Tea Seed Oil in Olive Oil		
Method No.	FSSAI 02.024:2021	**Revision No. & Date**	0.0
Scope	This test is useful to indicate the presence of tea seed oil.		
Caution	1. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 2. Sulphuric acid: Concentrated Sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If Sulphuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 3. Acetic Anhydride: It is a highly corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage. Breathing Acetic Anhydride can irritate the nose, throat and mouth. High concentrations can cause severe lung damage with coughing and/or shortness of breath. 4. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity.		
Principle	The test is based on the development of red color by acetic anhydride in the presence of sulphuric acid when a solution of oil in Chloroform is taken (Fitelson Test).		
Apparatus / Instruments	1.General glassware and apparatus (Refer 2.0 at page no. 1) 2.Test tubes – 150mm × 15 mm 3. Pipette – 2 mL, graduated to 0.1 mL 4. Dropper so calibrated that 7 drops of oil weigh 0.22 g 5. Water bath maintained at 50 °C		
Materials and Reagents	1. Chloroform 2. Concentrated Sulphuric acid 3. Acetic anhydride 4. Diethyl ether, anhydrous peroxide free, stored over Sodium		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Pipette into a test tube 0.8 mL of acetic anhydride, 1.5 mL of Chloroform and 0.2 mL of Sulphuric acid. 2. Cool to 5 °C and add approximately 0.22 g (7 drops) of oil. 3. If any turbidity appears, add acetic anhydride drop by drop with shaking until the solution becomes clear.		

	4. Keep at 5 °C for 5 min. 5. Add 10 mL of Diethyl ether previously cooled to 5 °C. 6. Stopper the test tube and mix thoroughly by inverting it twice. 7. Return the test tube to the bath at 5 °C. An intense red color which develops about a min after the addition of ether, reaches a maximum and disappears indicates pure tea seed oil. 8. A less intense color indicates presence of tea seed oil but caution must be exercised in interpreting results in the presence of olive oil.
Inference (Qualitative Analysis)	The test is generally applicable to mixture of olive oil and tea seed oil, but some olive oils yield a pink color and the test is therefore not reliable for the detection of less than 15% of tea seed oil in olive oil.
Reference	1. FAO Manuals of Food Quality Control 14 / 8, page 273 2. AOAC 17th edn, 2000, Official Method 936.12 Oil (Teaseed) in olive oil 3. AOCS, 6th edn, 2012. Official Method Cb 3-39; Codex Alimentarius – Recommended Method 24, 1970
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of Olive Residue (Pomace) Oil in Olive Oil		
Method No.	FSSAI 02.025:2021	**Revision No. & Date**	0.0
Scope	Pomace is the solid residue in olive oil industry and known as olive residue.		
Caution	1. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation.		
Principle	The test is based on the temperature of precipitation of salts of fatty acids after saponification.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Erlenmeyer flasks 3. Reflux condenser		
Materials and Reagents	1. Ethyl alcohol 2. Potassium hydroxide 3. Acetic acid		
Preparation of reagents	1. Alcoholic Potassium hydroxide: Potassium hydroxide (42.5 g) dissolved in 72 mL water made upto 500 mL with 95% ethyl alcohol. 2. Aqueous acetic acid: Mix 5 mL of acetic acid and 10 mL of water.		
Sample Preparation	The sample is filtered through paper at a temperature slightly above the melting point of certain solid constituents which could separate from the fluid fatty matter. Refer 3.0 at page no. 2		
Method of Analysis	1. Saponify 1 g of oil by boiling for 10 min with 5 mL alcoholic Potassium hydroxide. 2. After cooling add 1.5 mL aqueous acetic acid exactly, neutralizes 5 mL of aqueous alcoholic Potassium hydroxide and add 50 mL of 70% ethanol warmed to 50 °C. 3. Mix, insert a thermometer and allow to cool. 4. If a precipitate forms above 40 °C, the test for the presence of olive residue oil is positive. 5. Allow to cool to ambient temperature for 12 h. 6. Observe solution again. The formation of a flocculent precipitate floating in the middle of the liquid also indicates that the test is positive.		
Inference (Qualitative Analysis)	Cloudiness not forming into flakes does not indicate the presence of olive residue oil.		
Reference	1. Pearsons Composition and Analysis of Foods 9th edn, page 619 2. Codex Alimetarious Commission – recommended method 22 -1970		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

Alternative CODEX method Specific extinction in ultra-violet could be tested

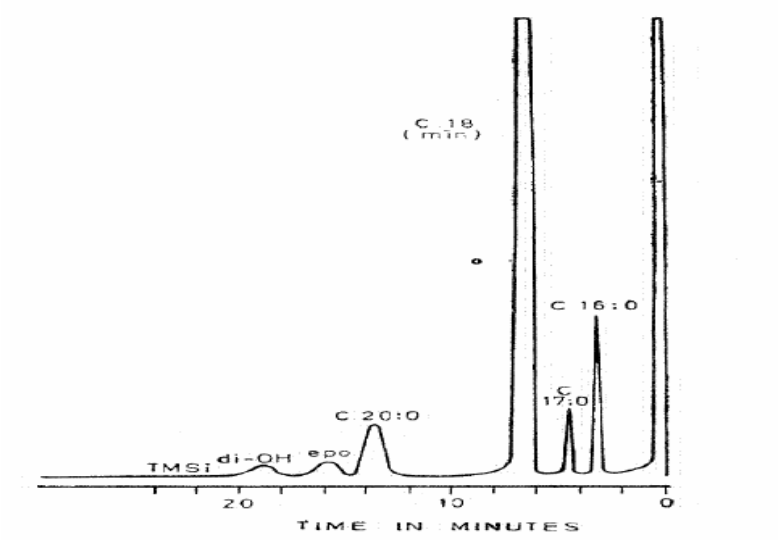
	Extinction (E) maximum at 232 nm	**Extinction (E) maximum at 270 nm**	**D E maximum variation at near 270 nm**
Virgin olive oil	3.50	0.30	[12]
Refined olive oil	-	1.10	0.16
Refined olive-pomace oil	6.00	2.00	0.20
Blends of refined olive oil and virgin olive oil	-	0.90	0.15
Blends of refined olive-pomace oil and virgin olive oil	5.50	1.70	0.18

	Test for Semi- Siccative Oil in Olive Oil		
Method No.	FSSAI 02.026:2021	**Revision No. & Date**	0.0
Scope	The binders of oil paints are known as siccative oils. One common measure of the "siccative" (drying) property of oils is iodine number, which is an indicator of the number of double bonds in the oil.		
Caution	1. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 2. Liquid bromine: Breathing bromine gas could causes cough, trouble breathing, gets a headache, causes irritation of mucous membranes (inside mouth, nose, etc.), dizzy, watery eyes. Getting bromine liquid or gas on skin could cause skin irritation and burns. The use of safe and suitable pipette i.e. Lunge-Ray pipette, is suggested for the handling and addition of bromine. 3. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane.		
Principle	The test is based on the reaction between semi- siccative (unsaturated) oils and bromine yielding substances, which form an insoluble precipitate at 0 °C.		
Apparatus	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Stoppered 50 mL Erlenmeyer flask 3. Bath of melting ice		
Materials and Reagents	1. Hexane or Petroleum ether (40-60 °C free from any residue) 2. Bromine liquid		
Preparation of reagents	Bromine solution prepared by adding drop by drop while shaking 4 mL of pure Bromine (the presence of Chlorine prevents the reaction) into100 mL of Hexane or Petroleum Ether chilled at 0 °C and kept in the melting ice bath until required.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. The oil to be tested is filtered and dried. 2. Place 1 mL of oil in a previously dried Erlenmeyer flask and dissolve in 10 mL Hexane. 3. Place the stoppered Erlenmeyer flask in the melting ice bath. 4. After 5 min add 10 mL of bromine solution in small quantities at a time while shaking and maintaining the temperature at 0 °C. 5. The color of the solution must clearly indicate excess of bromine. 6. Leave the Erlenmeyer flask in melting ice for 1 h, after which, note appearance of the solution. 7. If semi- siccative oil is present a flocculent precipitate will form varying in quantity according to the % of adulteration and the nature of adulterant oil.		
Inference (Qualitative Analysis)	The solution remains clear and transparent in the case of genuine olive oil.		
Reference	Codex Alimentarius Commission – Recommended method 21 – 1970		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of 9, 10 epoxy and 9, 10 dihydroxy stearic acid in Salseed Fat – Method A		
Method No.	FSSAI 02.027:2021	**Revision No. & Date**	0.0
Scope	Salseed fat contain 9, 10 epoxy and 9, 10 dihydroxy stearic acids Two methods have been prescribed namely 1. Gas Liquid Chromatography Method and 2. Preparatory TLC/HPTLC method The GLC method is to be used as a reference method.		
Caution	1. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 2. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 3. Methanol: Flammable liquid and vapor. May be fatal or cause blindness if swallowed. Cannot be made nonpoisonous. Harmful if inhaled or absorbed through skin. Causes irritation to skin, eyes, and respiratory tract. High vapor concentrations may cause drowsiness. May cause harm to the unborn child. Prolonged exposure may cause chronic effects. 4. Dichloromethane: Higher levels of dichloromethane inhalation can lead to headache, mental confusion, nausea, vomiting, dizziness and fatigue. Skin Exposure - Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns. 5. Sodium methoxide solution: Fire hazard - Highly flammable liquid and vapor. Irritating fumes and organic acid vapors may develop when material is exposed to elevated temperatures or open flame. Explosion hazard - May form flammable/explosive vapor-air mixture. 6. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 7. Pyridine: Pyridine can affect you when breathed in and by passing through your skin. Breathing Pyridine can irritate the nose and throat causing coughing and wheezing. Pyridine can cause nausea, vomiting, diarrhea and abdominal pain. Pyridine can cause headache, fatigue, dizziness, Pyridine may cause a skin allergy.		
Principle	**Gas Liquid chromatography method** The method consists of enrichment of triglycerides containing 1 mole of		

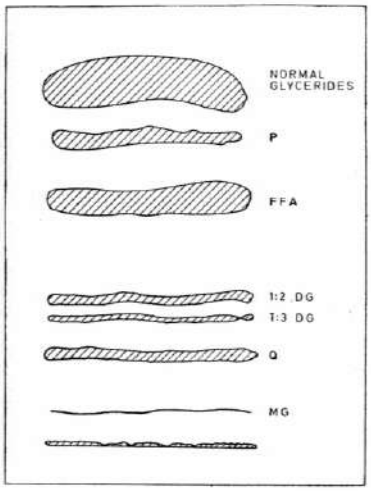
	9, 10 epoxy Stearic acid and 2 moles of predominantly Stearic acid (designated as P) and other triglyceride containing 9, 10 dihydroxy stearic acid in place of epoxy stearic acid (designated as Q) from a known mass of salseed fat by treatment with silicic acid in Hexane and desorbing these by a more polar solvent. The desorbate to which a known quantity of internal standard is added, is subjected to transmethylation and the methyl esters after silylation are analysed by GLC.
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Calcium chloride guard tubes 3. GC equipped with the required gases and FID detector 4 2.5 m×4 mm glass column packed with 1% OV17 on 80-100 mesh Gas Chrosom Q
Materials and Reagents	1. Hexane 2. Chloroform 3. Methanol 4. n – heptadeconoic acid methyl ester 5. Dichloromethane 6. Sodium methoxide solution 7. Nitrogen gas 8. Sodium chloride solution 9. Calcium Chloride (anhydrous) 10. Sodium sulphate (anhydrous) 11. Diethyl ether 12. Pyridine 13. Hexamethyldisilazane 14. Chlorotrimethylsilane
Preparation of reagents	1. 2M solution of sodium methoxide prepared using methanol 2. Saturated sodium chloride solution
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	**(1) Enrichment of 'P' and 'Q' and transmethylation:** 1. Dissolve 200 mg of refined salseed fat in 2 mL of n – hexane and stir with 600 mg of silicic acid (activated at 110 °C for 1 h before use) for 1.5 h using a magnetic stirrer. 2. Pipette out the supernatant Hexane and wash the residue with additional 2 mL of hexane and discard the hexane. 3. Add 2 mL of chloroform methanol (3:1 v/v) to the residual silicic acid and stir for 45 min. 4. Filter the contents and wash the residue with additional 2x2 mL chloroform- methanol (3: 1) mixture to ensure complete desorption of the adsorbed material. 5. Add a known amount of about 2 mg of n – heptadeconoic acid methyl ester to the combined chloroform- methanol filtrate contained in a 10 mL round bottom flask and evaporate solvent to dryness under a stream of nitrogen. 6. Dissolve the residue in 1 mL of dichloromethane and retreat it with 2 mL of 2M solution of sodium methoxide in methanol and keep at

	50 °C for 15 min with occasional shaking. 7. During this treatment connect the flask to a condenser and calcium chloride guard tube. 8. Dilute the contents with 2 mL of saturated sodium chloride solution and extract with n – hexane (3 x 2 mL) in a separating funnel. 9. Wash the combined hexane extract with water to ensure complete removal of alkali (by pH paper), dry over anhydrous sodium sulphate and evaporate to dryness under nitrogen. **(2) Silylation:** 10. Transfer the final residue to a 5 mL screw capped vial with the aid of ether and subsequently remove ether by evaporation under nitrogen. 11. Dissolve this in 0.2 mL of dry pyridine and treat with 0.1 mL of hexamethyldisilazane and 0.05 mL of chlorotrimethylsilane and keep at room temperature for 1 h. 12. Evaporate the reagents to dryness on a water bath at about 50 °C under nitrogen. **(3) GLC analysis:** 13. Dissolve the residue obtained above in hexane 14. Inject into GLC column. 15. The instrument should have a flame ionization detector and a 2.5 m x 4 mm glass column packed with 1% OV 17 on 80-100 mesh Gas Chrosom Q. 16. The temperature of oven and detector shall be 195°C. The carrier gas shall be Nitrogen with a flow rate of 60 mL/min and the chart speed shall be 25 cm/h
Calculation with units of expression	$P\% = \frac{\text{Peak Area of epoxy Acid Methyl ester}}{\text{Peak Area of the internal standard}} \times \text{Amt of internal standard (in mg)} \times \frac{904}{312} \times \frac{100}{\text{Mass of sample (in mg)}}$ $Q\% = \frac{\text{Peak Area of dihydroxy Methyl ester}}{\text{Peak Area of the internal standard}} \times \text{Amt of internal standard (in mg)} \times \frac{922}{330} \times \frac{100}{\text{Mass of sample (in mg)}}$ Note: 1. The GLC method of 'P' and 'Q' estimation is applicable to refined fats or fats with FFA less than 2%. In case of high FFA, fat neutralization should precede transesterification. 2. For this purpose spray a small quantity of fat with 3 N sodium hydroxide (10 % excess) containing 10 % sodium chloride at 50 – 60 °C under gentle stirring. 3. After allowing the soap to settle for a while, transfer the material to a tube and centrifuge. 4. Wash the supernatant oil free of soap and take for trans-esterification. Epoxy and dihydroxy fatty acids % by mass = $\frac{P+Q}{3}$

	 **Typical GLC Scan**
Reference	IS 7375 – 1979 Specification for Salseed fat
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of 9, 10 epoxy and 9, 10 dihydroxy stearic acid in Salseed Fat – Method B		
Method No.	FSSAI 02.028:2021	**Revision No. & Date**	0.0
Scope	Salseed fat contain 9, 10 epoxy and 9, 10 dihydroxy stearic acids		
Caution	1. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 2. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 3. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 4. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses.		
Principle	**Preparative Thin Layer chromatography method** 'P' and 'Q' are separated on preparatory TLC/HPTLC plates and the bands scraped, extracted with solvent, evaporated and weighed. The method is applicable to fats containing approximately 3% each or more of 'P' and 'Q'. Fats containing lesser proportion of 'P' and 'Q' need enrichment prior to preparative TLC/HPTLC in order to get reasonable amounts of 'P' and 'Q' for weighing. The method works well with refined fats or raw fats with F.F.A. upto 3 % or less but fats with high FFA should be refined as per Note under GLC method (25.1).		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Plate of 1 mm thick silica gel 3. TLC chambers 4. Thimbles 5. Whatman filter paper No 42		
Materials and Reagents	1. Silicic acid (suitable for lipid chromatography) 2. n-Hexane 3. Chloroform 4. Di ethyl ether 5. Acetic acid 6. Iodine crystals		
Preparation of reagents	Hexane, diethyl ether and acetic acid (60:40:1) as a solvent system.		

Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Weigh accurately about 10 g of salseed fat and dissolve in 100 mL of Hexane 2. Stir with 20 g of silicic acid (suitable for lipid chromatography activated at 110 °C for 1 h before use) for 4 h on a magnetic stirrer. 3. Filter the slurry using a Buchner funnel. 4. Distill the filtrate to obtain the normal triglycerides. 5. This fraction does not show the presence of 'P' and 'Q' when 2 mg of material is chromatographed on a TLC/HPTLC plate indicating that the 'P' and 'Q' are completely adsorbed. 6. It is necessary to do the TLC/HPTLC test to ensure absence of 'P' and 'Q' in the hexane extract and to arrive at the appropriate fat: silicic acid ratio. A ratio of 1: 1 is suitable. 7. Stir the residual silicic acid with 100mL of chloroform for 1 h and filter. Repeat the operation with fresh lot of 100 mL of chloroform. 8. Distill the combined filtrate to obtain the enriched fraction. **Thin Layer Chromatography** Dissolve a known mass (80- 90 mg) of the enriched fat in 0.5 mL of Chloroform. Apply as a streak on a preparatory plate of 1 mm thick silica gel and develop 4 such plates using hexane, ether and acetic acid (60:40:1) as a solvent system. Visualise the bands in an Iodine chamber and scrape the bands at R_f at 0.84 and 0.28 corresponding to 'P' and 'Q'. Transfer quantitatively into two separate thimbles and extract with chloroform in a Soxhlet. It takes about 1 h for extraction. After extraction, distill the chloroform. Transfer the residue carefully through a Whatman filter paper No 42 or equivalent to a tared 5 mL round –bottom flask using ether. Wash the filter paper thoroughly with ether and collect the washings in the same flask. Evaporate ether under a stream of Nitrogen and weigh the flask to a constant mass by keeping it in the oven at 105 °C.
Calculation with units of expression	Calculate the amount of 'P' and 'Q' in the original fat from the mass of the residue. Express the results as %age of epoxy and dihydroxy fatty acids by dividing the sum of 'P' and 'Q' by 3. **Note 1:**The specified solvent system (Hexane: Ether: Acetic acid (60:40:1) is satisfactory for resolving 'Q' but the resolution of 'P' from normal triglycerides is occasionally poor and appears to depend upon the activity of silica gel. The close Rf values of 'P'and normal triglycerides is likely to lead to errors in estimation of 'P'. In such cases a slightly less polar solvent (hexane: ether: acetic acid 80:20:1) will lead to a good resolution. 'P' and 'Q' should then be determined separately using appropriate solvent system. **Note 2:-**Generally the pattern of separation of various constituents in descending order of Rf on the plate is as follows:-

	i. Normal triglycerides ii. 'P' iii. FFA iv. 1,2 di-glycerides v. 1,3 di-glycerides vi. 'Q' vii. Monoglycerides  A typical chromatogram of Salseed Fat in Hexane; Ether; Acetic Acid (60:40:1)
Reference	IS 7375 – 1979 Specification for Salseed fat
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of Mineral Oil – Holde's method		
Method No.	FSSAI 02.029:2021	**Revision No. & Date**	0.0
Scope	Mineral oil is any of various colorless, odorless, light mixtures of higher alkanes from a mineral source, particularly a distillate of petroleum. Two methods are used to detect mineral oils in edible oils. Method A - Holde's test Method B - TLC/HPTLC test Method A is for rapid detection of mineral oil in vegetable oils and fats. It is sensitive when mineral oil is present to the extent of 1% or more. The test is not sensitive in the case of oils with high content of unsaponifiable matter. Method B shall be used where confirmation is required.		
Caution	Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed.		
Principle	Method A (Holde's Test) - The presence of mineral oil is indicated by the development of turbidity when hot distilled water is added to a freshly made alcoholic solution of the soap formed by the oil.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Conical flask (100 mL) with standard joint 3. Air condenser/Water Condenser to fit above		
Materials and Reagents	1. Oils and fats 2. Potassium hydroxide		
Preparation of reagents	Alcoholic Potassium hydroxide solution, 0.5 N		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Take 25 mL of the alcoholic KOH solution in a conical flask and add 1 mL of the sample of oil to be tested. 2. Boil on a water bath using an air- or water-cooled condenser till the solution becomes clear and no oily drops are found on the sides of the flask. 3. Take out the flask from the water-bath, transfer the contents to a wide mouthed warm test tube. 4. Add 25 mL of boiling distilled water along the sides of the test tube. Keep on shaking the tube lightly from side to side during the addition. 5. The turbidity indicates presence of mineral oil.		
Inference (Qualitative Analysis)	The depth of turbidity depends on the percentage of mineral oil present.		
Reference	1. Handbook of Food Analysis (Part XIII)-1984 Page 89 2. AOAC 17th edn, 2000, Official Method 945.102 – Oil (mineral) in fats – Qualitative Test		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Test for presence of Mineral Oil (Thin Layer Chromatographic test)		
Method No.	FSSAI 02.030:2021	**Revision No. & Date**	0.0
Scope	Mineral oil is any of various colorless, odorless, light mixtures of higher alkanes from a mineral source, particularly a distillate of petroleum.		
Caution	Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness.		
Principle	Being non-polar, mineral oils give faster moving spots on thin layer chromatographic plates, than the triglycerides.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Glass slides (7.6 × 2.5 cm) or glass plates of 20 x 5 cm or 20 x 10 cm may be used. 3. Developing tank. 4. Ultra-violet lamp (365 nm). This should be placed in a darkened enclosure		
Materials and Reagents	1. Silica-gel 'G' with calcium sulphate as binder (commercially available) 2. Petroleum ether 3. 2', 7'-dichloro-fluorescein 4. Ethanol		
Preparation of reagents	Spray reagent: 0.2% solution of 2', 7'-dichloro-fluorescein in 95% ethanol		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	**Thin Layer Chromatography** 1. Hold two slides together face to face and dip them in a slurry of silica gel G (45g) in a mixture of chloroform and methanol (80 + 20 mL). 2. Withdraw the slides, separate them and allow drying in air and activating at 110 °C for 15 min and cooling in a desiccator. 3. Apply 10 mL of a 10% solution of oil in chloroform on the glass slide/glass plate using a capillary tube. 4. Allow to dry and place the slide in a developing tank containing petroleum ether. Cover the tank and allow the solvent to travel for 6 cm from the origin (about 4 min). 5. Remove the plate from the tank, dry in air, spray with the fluorescein solution and view under UV light. 6. Appearance of a yellow fluorescent spot on the solvent front indicates the presence of mineral oil. 7. The vegetable oil forms a yellow streak about 2-3 cm long from the point of spotting.		
Inference (Qualitative Analysis)	If desired, a standard sample containing 1% by mass of liquid paraffin in a sample of pure oil under test may be prepared and tested simultaneously as reference sample.		
Reference	ISI Handbook of Food Analysis (Part XIII)-1984 Page 89		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Test for presence of Castor Oil		
Method No.	FSSAI 02.031:2021	**Revision No. & Date**	0.0
Scope	Triricinolein isolated from castor oil (*Ricinus communis*) is the triglyceride of ricinoleic acid. 'Triricinolein' a characteristic and predominate triglyceride component of castor oil.		
Caution	1. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 2. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane.		
Principle	'Triricinolein' is separated on silica gel TLC/HPTLC and visualized by iodine vapour.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1). 2. Slides: microscopic slides (7.6 ×1.5 cm) or glass plates of 20 ×5 cm or 20 ×10 cm may be used. 3. Developing tank: a tall beaker of at least 10 cm height/TLC/HPTLC developing chamber. 4. Visualization tank (Iodine chamber): A dry beaker or developing tank saturated with iodine vapour by placing a few crystals at the bottom and leaving for an hour.		
Materials and Reagents	1. Absolute Alcohol 2. Silica gel containing 15% Calcium sulphate as binder (silica gel G) passing 75 micron IS sieve 3. Hexane 4. Diethyl ether 5. Castor oil		
Preparation of reagents	1. Developing solvent: Hexane: Diethyl ether (1:1) 2. Standard castor oil solution – 1% castor oil dissolved in 100 mL absolute alcohol		
Sample Preparation	Refer 3.0 at page no. 2		

Method of Analysis	1. Coat microscopic slides or TLC / HPTLC plates with a slurry of silica gel G and water (1:2) with the help of an applicator. 2. Activate at 110 °C for one hour. Cool and keep in a desiccator. 3. Take 10 mL of suspected oil in a separating funnel. 4. Add 10 mL of absolute alcohol. Shake vigorously for one min and allow to separate the two layers. 5. Discard the lower oil layer and draw of the upper alcohol layer into a 25 mL beaker. 6. Concentrate alcohol extract to about 2 mL. **Thin Layer Chromatography** 7. Spot 10 µL of alcoholic extract and 10 µL of standard Castor oil solution on TLC/HPTLC plate. 8. Develop in developing tank containing Hexane: diethyl ether (1:1) upto 15 cm. 9. Air dry the plate and put in iodine chamber. 10. Occurrence of a spot at R_f of about 0.25 shows presence of castor oil. 11. All other spots will be above this. **Detection of Castor Oil in rancid oils** 12. The suspected rancid oil (5 mL) may be taken in a round bottom flask and treated with activated charcoal (2 g). 13. The contents are mixed thoroughly and heated on boiling water bath for about 30 min with constant shaking. 14. The bleached oil is filtered to separate the charcoal. 15. The filtered oil may now be passed through a mini column packed with neutral alumina (10 g) using hexane (50 mL) as eluent. 16. This bleached and neutralized oil may be spotted on the TLC/HPTLC plate for detecting presence of castor oil as above.
Inference (Qualitative Analysis)	1. The spot shall be noticed in the visualization tank since it fades on removing. This method has a sensitivity of one per cent. 2. This method is specific for castor oil, but rancid or oxidized oils give spots with the R_f values similar to those given by Castor oil. Hence, care should be taken when applying the TLC/HPTLC test to rancid oil and interpretation of result. In such cases the rancid oil has to be purified by "refining" as described above.
Reference	ISI Handbook of Food Analysis (Part XIII) –1984 Modified test for presence of Castor oil, page 91
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of Argemone Oil		
Method No.	FSSAI 02.032:2021	**Revision No. & Date**	0.0
Scope	Argemone (*Argemone mexicana L.*), yellow poppy, is a wild herb, which grows in mustard field and bears capsules full of brown black seeds. Because of its resemblance with black mustard, it is often used as an adulterant. The oil is reported to cause glaucoma, dropsy and sometimes total blindness due to the presence of alkaloids namely, sanguinarine and dihydrosanguinarine.		
Caution	1. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 2. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 4. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 5. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 6. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity.		
Principle	The hydrochloric acid extract of the oil sample containing argemone oil when subjected to TLC/HPTLC for separation of alkaloid gives fluorescent spot under UV light.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. TLC / HPTLC plates coated with silica gel G or precoated ready-made plates cut to suitable size. 3. Ultraviolet lamp (long wave – 366 nm) in a visualization chamber. 4. Pear-shaped flask.		
Materials and Reagents	1) Butanol 2) Acetic acid 3) Water		

	4) Hexane or Heptane 5) Acetone 6) Diethyl ether 7) Hydrochloric acid, cons. Sp. Gr. 1.19 8) Chloroform 9) Sodium hydroxide 10) Standard Argemone oil extract
Preparation of reagents	i) Solvent mixture (mobile phase) a) Butanol: Acetic acid: water 70:20:10 (v/v) b) Hexane or Heptane: Acetone 60:40 (v/v) ii) Chloroform: Acetic acid (90: 10 v/v) mixture iii) Aqueous sodium hydroxide solution 1 N
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Take 10 mL sample in a separating funnel and dissolve in 15 mL Diethyl ether. 2. Add 5 mL concentrated Hydrochloric acid and shake vigorously for 2 – 3 min. Allow to separate. 3. Contents of the separator funnel may be heated cautiously over the vent of heating water bath for some time for quick separation. 4. Transfer the acid layer to a 25 mL beaker. Place the beaker into a boiling water bath and evaporate till dryness. **Thin Layer Chromatography** 5. Dissolve the residue obtained after evaporation of hydrochloric acid in 1 mL of a mixture of chloroform and acetic acid (9:1). 6. Spot on TLC/HPTLC plate with the help of spotting capillary. Spot side by side standard Argemone oil extract (0.1% in ether). 7. Develop the plate in (a) Butanol: Acetic acid: water; or (b) Hexane: Acetone mixture. 8. Allow the solvent front to move up a distance of 10 cm and allow the plate to dry. 9. Place the plate under UV light in the visualization chamber. 10. Bright yellow or orange yellow fluorescent spots having R_f similar to the standard argemone oil will confirm presence of argemone oil. 11. The spot gives blue fluorescence under UV-light if plate is sprayed with 1% aqueous sodium hydroxide solution.
Inference (Qualitative Analysis)	The method is very sensitive and can detect argemone oil upto 50 ppm level.
Reference	Manual methods of Analysis for Adulterants and Contaminants in Foods ICMR (1990) page 12
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of presence of Karanja (*Pongamia glabra*) Oil		
Method No.	FSSAI 02.033:2021	**Revision No. & Date**	0.0
Scope	Seeds of *Pongamia glabra* contain oil, glabrin, karanjin, karanjone, pongaglabrone and pongamol along with other constituents.		
Caution	1. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 2. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 3. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 4. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation.		
Principle	Extraction of glabrin, karanjin, karanjone, pongaglabrone and pongamol using concentrated hydrochloric acid and their detection on TLC /HPTLC under ultra-violet light.		
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. All-glass separating funnel (100 mL capacity). 3. Measuring cylinder for separating funnel. 4. Wooden stand for separating funnel. 5. Hot water-bath. 6. Capillary tubes. 7. TLC/HPTLC plates (0.25 mm). Prepared by coating a slurry of silica gel G on glass plate of 10 × 20 cm diameter, activated at 110 °C for 1 h and stored in a desiccator. 8. Ultra-violet lamp long wave (366 nm) in a visualization chamber.		
Materials and Reagents	1. Hydrochloric acid AR Sp. Gr. 1.18 2. Petroleum ether 3. Diethyl ether 4. Glacial Acetic acid 5. Standard Karanja oil extracts		
Preparation of reagents	1. Solvent Mixture as mobile phase, petroleum ether: diethyl ether: acetic acid 60:40:1 (v/v) 2. Standard Karanja oil extracts (1.0% oil in any other oil extracted simultaneously with the sample)		

Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Take 20 mL of the suspected oil in a 100 mL capacity separating funnel. 2. Add 10 mL concentrated hydrochloric acid. 3. Shake the content gently, but consistently for 15 min. 4. Keep the separating funnel on a wooden stand for about 30 min to allow the separation of acid layer. 5. Draw out the acid layer in a glass beaker. Keep the beaker on a boiling water bath and evaporate the hydrochloric acid till dryness. **Thin Layer Chromatography** 1. Dissolve the residue in 0.5 mL of chloroform. 2. Spot the chloroform solution on a pre-activated TLC/HPTLC plate with the aid of capillary tube. 3. Spot standard Karanja oil extract side by side. 4. Develop the plate in solvent system petroleum ether: diethyl ether: acetic acid 60:40:1 v/v for 20 min. 5. Remove the plate, dry at room temperature and view under ultra-violet lamp. Appearance of three bluish green spots at R_f 0.34, 0.22 and 0.17 confirms the presence of Karanja oil.
Inference (Qualitative Analysis)	The test is sensitive to the extent of 0.01% Karanga oil.
Reference	Manual Methods of Analysis for Adulterants and Contaminants ICMR (1990) page 12
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of presence of hydrocyanic acid (Method A)		
Method No.	FSSAI 02.034:2021	**Revision No. & Date**	0.0
Scope	Hydrocyanic acid is sometimes present as an impurity in synthetic allyl-isothiocyanate which is commonly used as an adulterant to enhance the flavour of poor-quality mustard oil.		
Caution	1. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Lead acetate: Lead acetate is a very toxic substance. This substance is a potential carcinogen and a teratogen. It should not be handled by staff who are pregnant. It can be absorbed into the body through the skin or by inhalation or ingestion. 4. Ferric chloride: Ferric chloride can react with metals to form flammable and potentially explosive hydrogen gas. Toxic by ingestion. May cause irritation to the mouth and stomach.		
Principle	Two methods have been prescribed for the purpose of this test. Method A shall be used as referee method and method B as routine method. Method A - The hydrocyanic acid in the oil when heated over water bath is displaced by bubbling air and is absorbed in Potassium hydroxide solution. The cyanide is then tested with ferric chloride solution.		
Apparatus / Instruments	General glassware and apparatus (Refer 2.0 at page no. 1)		
Materials and Reagents	1.Potassium hydroxide 2. Lead acetate 3. Ferrous sulphate 4. Hydrochloric acid 5. Ferric chloride		
Preparation of reagents	1. Potassium hydroxide solution - approximately 2N 2. Lead acetate solution - approximately 2 N 3. Ferrous sulphate solution - approximately 2% 4. Ferric chloride solution - 20% (w/v) in water to which sufficient hydrochloric acid has been added to prevent hydrolysis.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Heat about 50 mL of the oil in a distillation flask by placing it on a water bath. 2. During heating pass through the oil for about 30 min, the air which has been purified by scrubbing through solution of Potassium hydroxide and lead acetate. 3. Connect the distillation flask to an absorption tube containing 5 mL of Potassium hydroxide solution. The air bubbling through the oil		

	carry with it the hydrocyanic acid and this is absorbed by the Potassium hydroxide solution. 4. Shake the solution with few drops of ferrous sulphate solution, acidify with few drops of hydrochloric acid and warm gently for 5 min. 5. Filter and add a few drops of ferric chloride solution.
Inference (Qualitative Analysis)	A blue or bluish-green color or precipitate in the solution indicates the presence of cyanide.
Reference	ISI Handbook of Food Analysis (Part XIII) – 1984, page 88
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of presence of hydrocyanic acid (Method B)		
Method No.	FSSAI 02.035:2021	**Revision No. & Date**	0.0
Scope	Hydrocyanic acid is sometimes present as an impurity in synthetic allyl-isothiocyanate which is commonly used as an adulterant to enhance the flavour of poor-quality mustard oil.		
Caution	1. Picric acid: Picric acid is toxic if swallowed, inhaled, or absorbed through the skin. Inhalation of dust may cause lung damage. Chronic exposure may cause liver or kidney damage. It is irritating to the skin and eyes and may cause an allergic skin reaction. 2. Tartaric acid: causes severe skin burns and eye damage. May cause respiratory irritation. If in eyes - rinse cautiously with water for several min. Remove contact lenses, if present and easy to do. 3. Sodium carbonate: eye contact can cause permanent corneal injury and possible burns. If you use sodium carbonate or soda ash in the household, take care to avoid ingestion or inhalation of dust. Due to these potential hazards, sodium carbonate should be handled with care		
Principle	Method B - This method is based on the reaction of hydrocyanic acid on picric acid paper which acquires a red color.		
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Whatman No.1 Filter paper		
Materials and Reagents	1. Picric acid 2. Filter paper (Whatman No. 1 or equivalent) 3. Tartaric acid 4. Sodium carbonate		
Preparation of reagents	1. Picric acid paper: Soak a filter paper (Whatman No. 1 or equivalent) in a saturated aqueous solution of picric acid, draining the excess liquid and drying the dyed paper in air. 2. Tartaric acid solution – 10% (w/v) 3. Sodium carbonate solution – 5% (w/v)		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Pour 30 mL of the oil into a 250 mL conical flask and mix well with about 50 mL of water. 2. Add 15 mL of 10% tartaric acid solution and mix. 3. Stopper the flask, with a velvet cork from which hangs a picric acid paper (about 7.5 cm long) previously wetted with a drop of 5% sodium carbonate solution. 4. The flask is placed on a hot water bath by the side of the steam vent and not directly on the steam for 30 to 45 min in presence of hydrocyanic acid, the picric acid paper acquires red color.		
Inference (Qualitative Analysis)	Ignore pink or light reddish hue which may, at times, appear at the periphery of the picric acid paper.		
Reference	ISI Handbook of Food Analysis (Part XIII) – 1984, page 88		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Test for presence of tricresyl phosphates and determination of tri-o-cresyl phosphate in edible oils (Method A)		
Method No.	FSSAI 02.036:2021	**Revision No. & Date**	0.0
Scope	Tricresyl phosphate (TCP), is an organophosphate compound that is used as a plasticizer and diverse other applications. It is a toxic substance that causes neuropathy.		
Caution	1. Isooctane: isooctane can affect when breathed in. Contact can irritate the skin and eyes. Repeated exposure can cause rash, dryness, and redness of the skin. Breathing isooctane can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath. Exposure can cause headache, nausea, reduced alertness, poor coordination, and feeling dizzy or lightheaded. Isooctane is a flammable liquid and a dangerous fire hazard. 2. Ethyl acetate: ethyl acetate is highly flammable, as well as toxic when ingestion or inhaled, and this chemical can be seriously damaging to internal organs in the case of repeated or prolonged exposure. Ethyl acetate can also cause irritation when it comes into contact with the eyes or skin. 3. Tricresyl phosphate: Tricresyl phosphate may burn, but does not readily ignite. Extinguish fire using an agent suitable for type of surrounding fire. Water may not be effective in fighting fires. Poisonous gases are produced in fire, including phosphorus oxides and phosphine.		
Principle	Tricresyl phosphate in contaminated edible oils is extracted using acetonitrile and detected by thin-layer chromatography as well as gas liquid chromatography.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Separatory funnels - 250 mL capacity. 3. TLC/HPTLC Plates - Prepare slurry of silica gel G with water (1:2 w/v) and spread over glass plates (0.325 mm layer on 20 x 20 cm plates) with applicator. Let the plates set at room temperature. Activate at 110 °C for 1 h, cool and store in a desiccator. 4. Gas Chromatograph - Fitted with flame ionization detector; stainless steel column (10' x 1/8') packed with 10% OV - 101 on 60 to 80 mesh Chromosorb-AW-DMCS; nitrogen carrier gas 30 mL/min, column temperature 250 °C, detector and injector temperature 300 °C; chart speed 1 cm/min. 5. Filter paper		
Materials and Reagents	1. Iso-octane 2. Ethyl acetate 3. 2, 6-dichloro-quinone chlorimide 4. A.R in absolute ethyl alcohol 5. Standard Tricresyl phosphate (TCP) 6. Tri-O-cresyl phosphate (TOCP)		
Preparation of reagents	1. Developing solvent - Iso-octane-ethyl acetate (90:10). Developing chamber lined with filter paper		

	2. Spray reagent: 0.5% solution of 2, 6-dichloro-quinone chlorimide. A.R in absolute ethyl alcohol (Gibbs reagent). Store reagent at 10 °C and use within 5 days.
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Take 10 mL oil sample containing Ca. 50 µg TCP or TOCP into separatory funnels. 2. Add 50 mL petroleum ether (40 – 60 °C) to dissolve the oil followed by 10 mL acetonitrile previously saturated with petroleum ether. Shake contents vigorously and let stand 10 min. 3. Collect lower acetonitrile layer in beaker and evaporate solvent on hot water bath. Dissolve residue in Ca. 1 mL ethyl or methyl alcohol. **Column Chromatography** 4. Inject about 1 mg (2.5 mg TCP / TOCP) of acetonitrile extract of the oil sample into GC apparatus; 5. Compare retention time and peak area of sample with that of standard T.C.P or T.O.C.P for quantitation. **Thin Layer Chromatography** 6. Thin layer chromatography: Spot ca 0.1 mL (Ca. 5 mg TOCP) of solution on TLC/HPTLC plate. 7. Develop plate in glass chamber containing iso-octane ethyl acetate (90:10) ca. 45 min to a height of 10 cm. 8. Remove plate and dry in air. Spray plate with Gibbs reagent and heat at 100 °C in oven. 9. Observe 15 min for characteristic blue-violet spot at R_f 0.27 corresponding to standard TCP or TOCP.
Inference (Qualitative Analysis)	Blue-violet spot at R_f 0.27 indicates the presence of TCP or TOCP
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Foods ICMR (1990) page 14
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of tricresyl phosphates and determination of tri-o-cresyl phosphate in edible oils (Method B)		
Method No.	FSSAI 02.037:2021	**Revision No. & Date**	0.0
Scope	Tricresyl phosphate (TCP), is an organophosphate compound that is used as a plasticizer and diverse other applications. It is a toxic substance that causes neuropathy.		
Caution	1. Isooctane: isooctane can affect when breathed in. Contact can irritate the skin and eyes. Repeated exposure can cause rash, dryness, and redness of the skin. Breathing isooctane can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath. Exposure can cause headache, nausea, reduced alertness, poor coordination, and feeling dizzy or lightheaded. Isooctane is a flammable liquid and a dangerous fire hazard. 2. Ethyl acetate: ethyl acetate is highly flammable, as well as toxic when ingestion or inhaled, and this chemical can be seriously damaging to internal organs in the case of repeated or prolonged exposure. Ethyl acetate can also cause irritation when it comes into contact with the eyes or skin. 3. Tricresyl phosphate: Tricresyl phosphate may burn, but does not readily ignite. Extinguish fire using an agent suitable for type of surrounding fire. Water may not be effective in fighting fires. Poisonous gases are produced in fire, including phosphorus oxides and phosphine. 4. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 5. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 6. Higher levels can cause trouble breathing, collapse and even death. Very high and repeated exposures may damage the liver. Repeated exposure to p-nitroaniline can cause a low blood count (anemia). P-nitroaniline is a reactive chemical and a dangerous explosion hazard.		
Principle	TLC/HPTLC Method based on alkaline hydrolysis of oil		
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Conical flask 250 mL capacity fitted with air condenser. 3. TLC plates 10x 20 cm or 20x 20 cm/HPTLC plates and a developing tank. 4. Sprayer 5. Air oven 6. Pipette 5 and 50 mL capacity 7. Capillary tubes		
Materials and Reagents	1. Potassium hydroxide 2. Aldehyde free alcohol		

	3. p-nitroaniline A.R 4. Hydrochloric acid 5. Sodium nitrite 6. Iso-octane 7. Ethyl acetate-AR 8. Tricresyl Phosphate Standard
Preparation of reagents	(1)Dissolve 70 – 80 g of KOH in an equal quantity of distilled water and add 2 L of aldehyde free alcohol. Allow to stand overnight, decant the clear liquid and keep in a bottle closed tightly with cork or rubber stopper (2)Alcoholic Potassium hydroxide solution 1.5 N. Add 8.5 g KOH in 100 mL of aldehyde free alcohol (3)Diazonium reagent - Dissolve 0.8 g p-nitroaniline A.R) in 250 mL lukewarm water. Add 20 mL of 20% Hydrochloric acid and mix properly to dissolve p- nitroaniline. Decant to remove any residual slick which remains. Cool and then add 50% Sodium nitrite solution until reagent is completely colorless. Store in a refrigerator. (4)Tricresyl Phosphate Standard- Prepare a 0.5% solution of tricresyl phosphate in pure rapeseed oil.
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Weigh accurately 5 g of sample in a conical flask and add 50 mL of alcoholic KOH. 2. Take 15 mL of standard TCP solution in another flask and add 50 mL of alcoholic KOH. 3. Fit both the flasks with air condenser and boil gently on water bath or hot plate for 1 h or till saponification is complete. **Thin Layer Chromatography** 4. Prepare a mixture of iso-octane ethyl acetate in the proportion of 90:10 as developing solvent. 5. Spot 10-20 µL of saponified sample as well as standard with a capillary tube. 6. Develop the plates in the solvent mixture for about 15 min so that the solvent front reaches 10 cm. 7. Dry the plates and spray with 1.5N alcoholic KOH. 8. Keep in the air oven at 60 °C. 9. Spray the plates with Diazonium reagent. 10. Appearance of red spot at the same R_f as the standard sample confirms the presence of tricresyl phosphate.
Inference (Qualitative Analysis)	Appearance of red spot confirms the presence of tricresyl phosphate.
Reference	IS Specification No. IS 548(Part II/ (Sec 22) 1993-Test for Detection of Tricresyl Phosphate in edible oils
Approved by	Scientific Panel on Methods of Sampling and Analysis

FSSAI FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of phosphorous in soya bean oil**		
Method No.	FSSAI 02.038:2021	**Revision No. & Date**	0.0
Scope	Crude soybean oil typically contains 1.5 to 2.5% phosphatide.		
Caution	1. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If Sulphuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 4. Sodium molybdate: It May cause eye, skin, and respiratory tract irritation. May be harmful if swallowed, inhaled, or absorbed through the skin.		
Principle	The method determines Phosphorous or the equivalent phosphatide by ashing in the presence of zinc oxide followed by spectrophotometer measurement of phosphorous as blue phosphomolybdic acid.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Platinum basins or crucibles suitable to withstand temperature of 600 °C. 3. Electric hot plate and muffle furnace. 4. Watch glass 75 mm diameter and Funnel short stem 50 mm diameter. 5. Filter paper – ashless, Whatman No 42 or equivalent, 90 mm diameter. 6. Volumetric flasks – 50 mL, 100 mL, 250 mL and 500 mL with glass stoppers. 7. Pipette – Mohr's type 10 mL with 0.1 mL subdivision. 8. Spectrophotometer with 1.0 cm cuvettes. For use in the visible region.		
Materials and Reagents	1. Concentrated Hydrochloric acid, sp. gr 1.18 2. Zinc oxide, reagent grade 3. Potassium hydroxide, reagent grade 4. Concentrated Sulphuric acid, sp .gr 1.84 5. Sodium molybdate, reagent grade 6. Hydrazine sulphate, reagent grade 7. Potassium dihydrogen phosphate, reagent grade dried for 2 h at 101°C		
Preparation of	1. Sodium molybdate - Carefully add 140 mL of concentrated sulphuric		

reagents	acid to 300 mL distilled water. Cool to room temperature and add 12.5 g of Sodium molybdate. Dilute to 500 mL with distilled water. Mix thoroughly and allow to stand for 24 h before use. (2) Hydrazine sulphate – 0.015% Dissolve 0.150 g hydrazine sulphate in 1 L water. (3) Potassium hydroxide – 50% solution Dissolve 50 g KOH in 50 mL distilled water (4) Standard Phosphate solution (a)Stock solution – Dissolve 1.0967 g of dry Potassium dihydrogen phosphate in distilled water and make up to 250 mL in a volumetric flask The solution contains 1 mg phosphorous per mL (b) Working Solution – Dilute 5 mL of standard stock solution with distilled water to 500 mL in a volumetric flask. This solution contains 0.01 mg phosphorous per mL.
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Weigh accurately 3 – 4 g of sample in a crucible or Pt basin. 2. Add 0.5 g Zinc oxide and heat slowly on the hot plate until the sample thickens, then gradually increase the heat until the mass is completely charred. 3. Place in a muffle furnace at 550 – 600 °C and hold for 2 h. 4. Remove and cool to room temperature. 5. Add 5 mL distilled water and 5 mL Hydrochloric acid to the ash. 6. Cover the crucible with a watch glass and heat gently to boiling for 5 min. 7. Filter the solution in a 100 mL volumetric flask. Wash the inside of the watch glass and the crucible with about 5 mL of hot water using a wash bottle with a fine stream of water. Wash the crucible and filter paper with 4 additional portions of hot distilled water. 8. Cool the solution to room temperature and neutralize to a faint turbidity by drop-wise addition of 50% KOH solution. 9. Add concentrated Hydrochloric acid drop-wise until the precipitate is just dissolved, then add 2 additional drops. 10. Dilute to volume with water and mix thoroughly. 11. Pipette 10 mL of this solution into a clean, dry 50 mL volumetric flask. 12. Add 8 mL of hydrazine sulphate solution and 2 mL of sodium molybdate solution in this order. 13. Stopper and invert 3 – 4 times. 14. Loosen the stopper and heat for 10± 0.5 min in a vigorously boiling water bath. 15. Remove from bath, cool to 25±5 °C in a water bath, dilute to volume and mix thoroughly. 16. Transfer the solution to a clean dry cuvette and measure the absorbance at 650 nm in a spectrophotometer adjusted to read 0% absorbance (100% transmittance) for distilled water. 17. Prepare a reagent blank without the oil test sample. Measure the phosphorus content of the sample and the blank by comparison

	with the standard curve. 18. Preparation of standard curve-Pipette 0.0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mL of standard working solution into 50 mL volumetric flasks. Dilute each to 10 mL with distilled water using a measuring pipette. 19. Add hydrazine sulphate and sodium molybdate as above. Plot the absorbance of each standard against its phosphorous content in mg on a linear graph paper.
Calculation with units of expression	Phosphorous$= \frac{10\times(A-B)}{W\times V}$ Where A = Phosphorous content of sample aliquot in mg B = Phosphorous content of the blank aliquot in mg W = Weight of sample in g V = Volume of solution taken for color development Note: - Phosphorous content can also be determined by Atomic Absorption Spectrophotometer at a wave length of 213 nm following other instrumental parameters.
Reference	AOCS (1989) Official Method Ca 12 -55, Phosphorous
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Nickel in Vanaspati		
Method No.	FSSAI 02.039:2021	**Revision No. & Date**	0.0
Scope	Nickel can be determined by spectrophotometric method as well as by Atomic Absorption Spectrophotometer using graphite furnace.		
Caution	1. Liquid bromine: Breathing bromine gas could causes cough, trouble breathing, gets a headache, causes irritation of mucous membranes (inside mouth, nose, etc.), dizzy, watery eyes. Getting bromine liquid or gas on skin could cause skin irritation and burns. The use of safe and suitable pipette i.e. Lunge-Ray pipette, is suggested for the handling and addition of bromine. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Dimethylglyoxime: It may cause eye and skin irritation. It may cause respiratory and digestive tract irritation. It may be harmful if swallowed. Combustible solid, slightly toxic by ingestion. 4. Nickel sulfate: Nickel sulfate is a carcinogen--handle with extreme caution. Breathing nickel sulfate can irritate the nose, throat and lungs causing cough, phlegm and shortness of breath. Nickel sulfate may also cause a skin allergy. If allergy develops, very low future exposure can cause itching and a skin rash.		
Principle	Nickel dimethyl glyoxime complex (red color) is prepared and it is estimated quantitatively using spectrophotometry.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Platinum dish 3. Muffle furnace		
Materials and Reagents	1. Concentrated Hydrochloric acid 2. Bromine liquid 3. Dimethly glyoxime 4. Ethyl alcohol 5. Nickel sulphate (A.R, 99.9% Pure)		
Preparation of reagents	1. Saturated Bromine Water 2. Dimethly glyoxime (0.1%) solution in 95% alcohol		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. The spectrophotometric method involves burning of 20 – 25 g of vanaspati in a platinum dish on a low flame. 2. Ash the remaining residue in muffle furnace at 500 °C 3. Dissolve the ash in about 5 mL of concentrated Hydrochloric acid. 4. Evaporate excess acid to dryness to obtain residue. 5. Dissolve the residue in water and making upto a known volume. 6. An aliquot of the solution (5 - 10 mL) is taken in a 25 mL		

	volumetric flask. 0.5 mL saturated Bromine water is added. 7. Allow to stand for 1 min, followed by addition of 1 ml of ammonia and 2 mL of 0.1% dimethyl glyoxime solution in 95% alcohol and mixed. 8. The final volume is made up to 25 mL with alcohol. 9. The 'absorbance maxima' is recorded at 445 nm within 10 min of addition of the dimethyl glyoxime solution. 10. Preparation of calibration graph - A standard stock solution of Nickel is prepared separately by dissolving 2.2617 g of Nickel Sulphate (A.R, 99.9% Pure) in 30 mL of concentrated Hydrochloric acid and making up the volume to 500 mL with distilled water. 11. This solution contains 1000 µg Ni/mL Working standards are prepared by diluting the stock solution to give 0.1 – 1.0 µg/mL Nickel. 12. A calibration graph is prepared with different working standards.
Calculation with units of expression	The amount of nickel in the sample is extrapolated from the standard graph.
Reference	1. Prakash and Sarin(1991) J. Fd Sci. Technol., 28 (1) 42-43. 2. AOAC 17th edn 2000, Official Method 990.05 Copper, Iron and Nickel in Edible Oils and Fats, Direct Graphite Furnace AAS Method.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method for qualitative test for Vitamin A in Vanaspati: Antimony trichloride method		
Method No.	FSSAI 02.040:2021	**Revision No. & Date**	0.0
Scope	Vitamin A is fortified in vanaspati made from oils.		
Caution	1. Antimony trichloride: Causes severe skin burns and eye damage. It May cause respiratory irritation. Toxic to aquatic life with long lasting effects. Do not breathe dust. 2. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses.		
Principle	The melted sample is treated with antimony trichloride and observed for appearance of blue coloration, which indicates presence of Vitamin A.		
Apparatus / Instruments	General glassware and apparatus (Refer 2.0 at page no. 1). Test tube.		
Materials and Reagents	1. Antimony trichloride 2. Chloroform 3. Anhydrous calcium chloride		
Preparation of reagents	Antimony trichloride Solution: Prepare by dissolving 113.4 g antimony trichloride in 300 to 400 mL of chloroform. Add 5 g of anhydrous calcium chloride and filter while hot. Dilute the filtrate to 500 mL with chloroform.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Take10 mL of antimony trichloride solution in a test tube and add 15 mL of melted Vanaspati. 2. The material shall be considered to have passed the test if a blue coloration appears immediately at the interface, indicating the presence of VitaminA. *Notes* 1 *Antimony trichloride solution is made in chloroform and this phase is heavier than Vanaspati. Therefore, Vanaspati should be added to antimony trichloride solution.*		
Inference (Qualitative Analysis)	Special care should be taken in carrying out this test since the reaction is spontaneous and the blue color developed is very unstable.		
Reference	An alternative Spectrophotometric method: IS Specification 5886: 1970		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of carotenoid content of raw palm oil		
Method No.	FSSAI 02.041:2021	**Revision No. & Date**	0.0
Scope	Oil palm is the largest source of natural carotenes. There are 500–700 ppm of carotenes in crude palm oil (CPO) and 4,000–6,000 ppm in the oil obtained from the palm-pressed fiber, a by-product from the oil palm fruits milling.		
Caution	Cyclohexane: Danger! Extremely flammable liquid and vapor. Vapor may cause flash fire. Harmful or fatal if swallowed.		
Principle	The absorption of a solution of the fatty material in cyclohexane is measured at 445 nm. The percentage content of total carotenoids (m/m) is calculated as beta carotene.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1). 2. Spectrophotometer capable of measurement at 445 nm and using matched or paired parallel sided glass or silica cells of 1 cm path. 3. Volumetric flask 100 mL		
Materials and Reagents	1. Oils and fats 2.Cyclohexane - spectroscopic grade		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Weigh to the nearest 1 mg between 0.5 and 1.0 g of the oil into a 100 mL volumetric flask. 2. Dissolve the oil in cyclohexane and make upto mark. 3. Fill a 1 cm glass or silica cell with the solution of the oil and fill a second matched cell with cyclohexane. 4. Take absorption readings in the spectrophotometer at 445 nm. 5. If necessary, dilute the original solution to a measured volume and take further readings so that the observed absorptions are between 0.2 and 0.8 optical density.		
Calculation with units of expression	Carotene content (mg/kg as beta-carotene) $= \frac{383\ \mathrm{E}}{\mathrm{t}\times\mathrm{c}}$ Where, E = Observed difference in absorption between sample solution and cyclohexane t = path length of the cell c = concentration used for absorption measurement		
Reference	British Standard Methods of Analysis – BS 684, section 2.2:1977 Determination of carotene in vegetable oils		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Method for presence of rancidity		
Method No.	FSSAI 02.042:2021	**Revision No. & Date**	0.0
Scope	Rancidity is the characteristic, unpalatable odour and flavour of edible fats and oils following oxidative or hydrolytic degradation. When edible oil is stored for a long time it undergoes oxidation and becomes rancid. Fats and oils have carbon - carbon double bond in their structure. This process can occur in raw foodstuffs, refined or used edible oils and processed foods containing edible oils.		
Caution	1. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 2. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 3. Potassium iodide: Common side effects of Potassium Iodide include: Allergic reactions (skin rashes such as hives; swelling of various parts of the body such as the face, lips, tongue, throat, hands or feet; fever with joint pain, trouble breathing, speaking or swallowing, wheezing, or shortness of breath). 4. Sodium thiosulphate: Sodium thiosulphate is moderately toxic when ingested. Remove contaminated clothing and wash the affected area on the skin with soap or mild detergent and large amounts of water until all evidence of the chemical has been removed (approximately 15 min). Wash contaminated clothing before reuse. 5. Potassium dichromate: Corrosive. Causes severe burns to every area of contact. Harmful if swallowed or inhaled. Affects the respiratory system, liver, kidneys, eyes, skin and blood.		
Principle	In routine work apart from the free fatty acid determination, the analysis should include the determination of peroxide value, Kries test and ultra-violet absorption at 234 nm and 268 nm to establish rancidity. Peroxide value is an indication of the extent of oxidation and rancidity suffered by oil.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Mohr's pipette		
Materials and Reagents	1. Oils and Fats 2. Acetic acid 3. Chloroform 4. Potassium iodide 5. Sodium thiosulphate 6. Potassium dichromate 7. Starch		

Preparation of reagents	1. Acetic acid - chloroform solvent mixture (3: 2). Mix 3 volumes of glacial acetic acid with 2 volumes of chloroform. 2. Freshly prepared saturated potassium iodide solution. 3. Sodium thiosulphate (0.1 N and 0.01 N) solutions. Weigh 25 g of sodium thiosulphate and dissolve in 1000 mL of distilled water. Boil and cool, filter if necessary. Standardize against standard potassium dichromate solution. 4. Starch solution - 1% water-soluble starch solution
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Weigh 5 g (±50 mg) sample into a 250 mL stoppered conical flask. 2. Add 30 mL acetic acid chloroform solvent mixture and swirl to dissolve. 3. Add 0.5 mL saturated potassium iodide solution with a Mohr's pipette. 4. Let stand for one min in dark with occasional shaking, and then add about 30 mL of water. 5. Slowly titrate the liberated iodine with 0.1 N sodium thiosulphate solution with vigorous shaking until yellow color is almost gone. 6. Add about 0.5 mL starch solution as indicator and continue titration shaking vigorously to release all I_2 from chloroform layer until blue color disappears. 7. If less than 0.5 mL of 0.1 N sodium thiosulphate is used repeat using 0.01 N sodium thiosulphate. Conduct blank determination (must be less than 0.1 mL 0.1 N sodium thiosulphate).
Calculation with units of expression	Peroxide value expressed as milliequivalent of peroxide oxygen per kg sample (meq/kg): $$\text{Peroxide value} = \frac{\text{Titre} \times \text{N} \times 1000}{\text{Wt of sample}}$$ Where, Titre = mL of Sodium Thiosulphate used (blank corrected) N = Normality of sodium thiosulphate solution. Fresh oils usually have peroxide values well below 10 meq/kg. A rancid taste often begins to be noticeable when the peroxide value is above 20 meq/kg (between 20 – 40 meq/Kg). In interpreting such figures, however, it is necessary to take into account the particular oil or fat.
Reference	1. AOAC 17th edn, 2000, Official Method 965.33 Peroxide Value in Oils and Fats. 2. Pearsons Composition and Analysis of Foods 9th edn page 641.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Rancidity - Kries Test		
Method No.	FSSAI 02.043:2021	**Revision No. & Date**	0.0
Scope	Rancidity is the characteristic, unpalatable odour and flavour of edible fats and oils following oxidative or hydrolytic degradation.		
Caution	1. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 4. Trichloroacetic acid 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.		
Principle	Rancidity can be determined qualitatively and quantitatively by Kries test.Among the chemical methods, Kreis test is a promising one for early detection of rancidity, particularly aldehydes with a characteristic odour impact. The color development in the test is critical and requires optimization.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Lovibond cell 3. Color glasses 4. UV-Vis Spectrophotometer		
Materials and Reagents	1. Phloroglucinol 2. Diethyl ether 3. Concentrated hydrochloric acid 4. Trichloroacetic acid 5. Glacial acetic acid		
Preparation of reagents	1. Phloroglucinol (0.1%) solution in diethyl ether. 2. Phloroglucinol (1%) solution in glacial acetic acid. 3. Trichloroacetic acid (30%) solution in glacial acetic acid.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	**Qualitative** 1. Shake 5 mL of the oil vigorously with 5 mL of 0.1% phloroglucinol solution in diethyl ether		

	2. Add 5 mL of concentrated hydrochloric acid. A pink color indicates incipient rancidity. **Quantitative – Method** 3. Weigh 0.8 – 1.02 g of oil or fat into a 100 mL beaker. 4. Melt sample of fat. 5. Add slowly with stirring 20 mL of phloroglucinol (0.1 g in100 mL of diethyl ether, freshly prepared) until sample dissolved. 6. Transfer solution to a separating funnel, add 10 mL concentrated Hydrochloric acid, shake well and allow to separate. 7. Run off acid layer into a 1inch (2.54 mm) Lovibond cell and match the color using red, yellow and blue glasses. 8. Express result as red Lovibond units. Upto 3 red units indicates incipient rancidity, between 3 and 8 units indicates the end of induction period, over 8 units indicates definite rancidity. **Quantitative – Method 2** 1. Shake 5 mL of oil and 5 mL chloroform in a stoppered test tube. 2. Add 10 mL of a 30% solution of trichloroacetic acid in glacial acetic acid and 1 mL of 1% solution of phloroglucinol in glacial acetic acid. 3. Incubate the test tube at 45 °C for 15 min. 4. After incubation, add 4 mL of ethanol and immediately measure the absorbance at 545 nm.
Inference (Qualitative Analysis)	Absorbance values below 0.15 indicate no rancidity. Absorbance values greater than 0.2 denote incipient rancidity and absorbance values around 1.0 show that the sample is highly rancid.
Reference	1. Pearsons Composition and Analysis of Foods 9th edn, page 642 2. Manual Methods of Analysis for Adulterants and Contaminants ICMR (1990) page 16.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Rancidity – UV method		
Method No.	FSSAI 02.044:2021	**Revision No. & Date**	0.0
Scope	Rancidity is the characteristic, unpalatable odour and flavour of edible fats and oils following oxidative or hydrolytic degradation.		
Caution	Iso-octane: breathing isooctane can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath. Exposure can cause headache, nausea, reduced alertness, poor coordination, and feeling dizzy or light headed. Isooctane is a flammable liquid and a dangerous fire hazard		
Principle	Oxidized fatty acids containing conjugated double bonds absorb UV strongly between 230 and 375 nm, dienes absorbing at 234 nm and trienes at 268 nm. Conjugated trienes may be formed by industrial processing, e.g. decolorising with bleaching earths. A secondary absorption by trienes occurs at about 278 nm. In the early stages of oxidation, the UV absorption increases somewhat proportionately to the uptake of oxygen and the formation of peroxides. The UV absorption curve forms plateau just before the end of the induction period. The magnitude of UV absorbance is not readily related to the amount of oxidation; so the method is best applicable to detecting relative changes in oxidation of oil in comparison experiments or stability tests.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. UV spectrophotometer		
Materials and Reagents	1. Oils and Fats 2. Iso-octane		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Weigh accurately into a 25 mL volumetric flask, an amount of the oil sample so that the absorbance of its solution in iso-octane in a 10 mm quartz cell lies between 0.2 and 0.8. 2. Trace the absorption curve against iso-octane between 220 and 320 nm and select the wavelength (λmax) of maximum absorption near 230, 268 and 278 nm, and the absorbance (A) at these points.		
Calculation with units of expression	The specific absorbance $E_{1\ cm}^{1\%} = A \times c \times d$ Where, A=Absorbance 'c' is the concentration of the sample solution (g/100 mL) 'd' is the cell length in cm.		
Reference	1. Pearson's Composition and Analysis of Foods 9th edn, page 643. 2. Manual Methods of Analysis for Adulterants and Contaminants ICMR (1990) page 16.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Method for isolation and identification of oil soluble colors		
Method No.	FSSAI 02.045:2021	**Revision No. & Date**	0.0
Scope	Oil soluble colors are natural as well as synthetic which are soluble in oils and fats. A few acid soluble and base soluble colors are added to oil.		
Caution	1. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 2. Basic aluminum oxide: May cause irritation to skin, eyes, and respiratory tract. Hazard is principally that of a nuisance dust. Coughing or shortness of breath may occur in cases of excessive inhalation. 3. Benzene: Benzene has long been recognized as capable of increasing the risk of leukemia and other blood disorders, and benzene can damage blood-forming cells in the bone marrow. 4. Ammonia solution: Contact with concentrated ammonia solutions may cause corrosive injury including skin burns, permanent eye damage or blindness. The full extent of eye injury may not be apparent for up to a week after the exposure. Contact with liquefied ammonia can also cause frostbite injury. 5. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 6. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 7. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 8. Ethyl acetate: ethyl acetate is highly flammable, as well as toxic when ingestion or inhaled, and this chemical can be seriously damaging to internal organs in the case of repeated or prolonged exposure. Ethyl acetate can also cause irritation when it comes into contact with the eyes or skin. 9. Antimony trichloride: Causes severe skin burns and eye damage. It May cause respiratory irritation. Toxic to aquatic life with long lasting effects. Do not breathe dust.		
Principle	The fat in the unaltered state or extracted from the foodstuff, is dissolved in petroleum ether. The solution is subjected to chromatography on a column of Aluminium oxide and the coloring matters undergo elution by		

	means of several elution solvents. The eluates are evaporated to dryness under vacuum and the residues subjected to saponification, if need be, are taken up in diethyl ether and identified using benzene as solvent.
Apparatus/ Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Balance 3. Aluminium dish – diameter 7 cm 4. Drying chamber, set at 60 °C 5. Soxhlet apparatus 6. Graduated Test tubes – 10, 25, 50 100, 250 mL 7. Chromatography Tube – 20 cm × 1 cm diameter with a tap 8. Round bottom flask 100 mL with ground glass joint 9. Rotary evaporator 10. Development tank for holding TLC plates of 20 × 20 cm 11. TLC plates – 20 × 20 cm coated with silica gel G to a thickness of 0.25 mm/HPTLC plates 12. Microcapillary pipettes of 2 µL or equivalent 13. Oven – set at 100°C
Materials and Reagents	1. Sea sand 2. Ethanol 3. Petroleum ether 40 – 60 °C 4. Basic Aluminium oxide 5. Benzene 6. Acetone 7. Petroleum Ether 8. Ammonia 25% (m/m), density - 0.910 9. Potassium hydroxide 10. Reference colors 11. Chloroform 12. n – hexane 13. Ethyl acetate 14. Antimony trichloride
Preparation of reagents	1. Sea sand – washed in hydrochloric acid and calcined 2. Ethanol 95% (v/v) 3. Basic Aluminium oxide- activated for 1 h at 400 °C 4. Mixture of Petroleum Ether and acetone 98:2. Measure exactly by pipetting 2 mL of petroleum ether from a filled 100 mL flask and replace it with 2 mL of acetone 5. Mixture of Petroleum ether and acetone 1:1 (v/ v). Measure 25 mL of pet. ether and 25 mL of acetone and mix 6. Mixture of ethanol and acetone 4:1 (v/v). Measure 40 mL of acetone and 10 mL of ethanol and mix 7. Mixture of ethanol and ammonia 2:1 (v/v). Measure 40 mL of ethanol and 20 mL of ammonia 0.910 and mix 8. Ethanolic Potassium. Hydroxide – 0.5 M Weigh 14 g Pot hydroxide and dissolve in 500 mL ethanol. Keep in dark 9. Solutions of reference colors – 0.5% in ethanol or Chloroform. Dissolve 50 mg of each reference color in 10 mL of ethanol except

	carotene which must be dissolved in chloroform. 10. Mixture of n – hexane and ethyl acetate, 9:1 (v/v). 11. Carr– Price reagent – Dissolve 25 g of antimony trichloride in 75 mL of chloroform in a glass stoppered conical flask.
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	1. Extraction from foodstuff 2. Weigh 5 – 10 g sample in an aluminium dish containing sand. 3. Add 5 - 10 mL ethanol and leave mixture in oven overnight. 4. Transfer contents of dish to a thimble or filter paper and extract for 4 h in a Soxhlet. 5. Evaporate the solvent from the extract and take residue in 10 mL of petroleum ether in a beaker. 6. Extraction from oil 7. Dissolve 0.5 - 1 g oil in10 mL petroleum ether. 8. Place a plug of cotton wool in the chromatography tube and push this down to just above the tap. 9. Fill the tube with a suspension of aluminium oxide in benzene so as to obtain a column of 10 cm in height. 10. Run off benzene taking care that the column does not become dry. 11. Rinse the column with 50 mL of petroleum ether or until all benzene has been removed. 12. Pour the petroleum ether extract of the color obtained above on to the column. 13. Regulate the speed of the flow to about 1 mL/min. 14. Rinse the column with 100 mL of Petroleum ether. Do not allow the column to become dry. Discard the eluate. 15. Eluate Carotenes with 50 mL of mixture of petroleum ether/acetone. 16. Collect eluate in a 100 mL round bottom flask. 17. Evaporate under partial vacuum using a rotary evaporator or a current of nitrogen with the flask over a water bath. 18. Take up residue in 1 mL diethyl ether. 19. Eluate the amino- aniline colors with 50 mL of mixture of petroleum ether /acetone 1:1. 20. Collect eluate in 100 mL flask, evaporate under partial vacuum using rotary evaporator or by current of nitrogen with flask over a waterbath. 21. Take up residue in 1 mL diethyl ether. 22. Elute the hydroxyl aniline color with 50 mL of acetone / ethanol mixture. Collect eluate in a 100 mL flask. 23. Evaporate to dryness under vacuum using rotary evaporator or on a water bath in a current of nitrogen. 24. Take up residue in 1 mL of diethyl ether. 25. Elute the bixin and the hydroxyl aniline colors which may still remain on the column with 50 mL of the mixture of ethanol / ammonia 2:1. 26. Collect the eluate in a 100 mL round bottomed flask. 27. Evaporate under partial vacuum using a rotary evaporator or in a

	current of nitrogen with the flask on a water-bath. 28. Take up residue in 1 mL of diethyl ether. 29. Change of color of the aluminium column to a red violet shade after the ethanol / ammonia mixture has been added indicates presence of curcumin in the sample. 30. The presence of residual oil or fat in the eluted colors can hinder identification and it is desirable to saponify the lipids present. 31. Add 50 mL of ethanolic Potassium hydroxide solution and some fragments of pumice stones. 32. Boil for 45 min under reflux. 33. Cool and transfer solution to a separating funnel using 100 mL water. 34. Carefully extract the aqueous phase, if it does not contain bixin once with 50 mL and twice with 25 mL diethyl ether. 35. Then wash the ethereal extracts three times using 25 mL water each time. 36. If it contains bixin acidify with sulphuric acid 4 M and extract once with 50 mL and twice with 25 mL diethyl ether. 37. Wash ethereal extracts 3 times with 25 mL water each time. 38. Dry the ether phase with anhydrous magnesium sulphate; evaporate under partial vacuum in a rotary evaporator or in a current of nitrogen over a water bath. 39. Take up residue for identification. Thin Layer Chromatography -Identification 1. Spot 4 µL or more of each of the solutions using a microlitre pipette about 2.5 cm away from the edge of the plate. Space the spots at an interval of 2 cm. 2. In the same way spot 2 µL of solutions of reference colors. 3. Develop plate with benzene in a developing tank saturated with the vapours of the solvent, allow to migrate over a distance of 17 cm. 4. Allow the plate to dry in air. 5. Develop again with benzene if necessary. 6. To separate Sudan I from Sudan II develop with mixture of n – hexane / ethyl acetate. 7. Examine the plate and identify the colors comparing the Rf values of spots of extracts with R_f values of the reference colors. 8. After examination, place the plate in a tank containing enough Carr–Price reagent to saturate the tank with its vapour until the plate becomes visibly wet. 9. A blue stain appearing in the fraction obtained with ethanol /ammonia 2:1 indicates presence of bixin. 10. Heat the plate for 10 min at 100 °C. The blue stain turns reddish brown.
Inference (Qualitative Analysis)	Different colors are resolved in TLC and observed according to their Rf values
Reference	1. FAO Manuals of Food Quality Control 14/ 2, page 69.

	2. Pearsons Composition and Analysis of Foods, 9th edn, 1991, page 107.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of test for presence of synthetic oil soluble colors		
Method No.	FSSAI 02.046:2021	**Revision No. & Date**	0.0
Scope	Oil soluble colors are natural as well as synthetic which are soluble in oils and fats.		
Caution	1. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases.		
Principle	Hydrochloric acid test - The petroleum ether solution of oil sample gives different shades of color with different concentrations of hydrochloric acid in presence of coal tar synthetic oil soluble color in the oil/fat.		
Apparatus / Instruments	General glassware and apparatus (Refer 2.0 at page no. 1)		
Materials and Reagents	1. Concentrated hydrochloric acid 2. Petroleum ether		
Preparation of reagents	Concentrated hydrochloric acid – Prepare 4:1, 3:1, 2:1 and 1:1 hydrochloric acid: water mixture.		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. To 5 mL of oil sample in separate test tubes add 15 mL of petroleum ether followed by 5 mL of hydrochloric acid of different concentrations to different tubes. 2. Observe for the change in the color indicating the presence of synthetic oil soluble color in the sample.		
Inference (Qualitative Analysis)	Change in the color of oil indicates the presence of synthetic colors soluble in oils		
Reference	ISI Handbook of Food Analysis, IS 548, 1976, part II.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Thin layer chromatography method for isolation and confirmation of oil soluble colors		
Method No.	FSSAI 02.047:2021	**Revision No. & Date**	0.0
Scope	Oil soluble colors are natural as well as synthetic which are soluble in oils and fats.		
Caution	1. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 2. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 3. Benzene: Benzene has long been recognized as capable of increasing the risk of leukemia and other blood disorders, and benzene can damage blood-forming cells in the bone marrow. 4. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation.		
Principle	The oil sample in hexane is treated with silica gel to absorb the colors. After eluting the oil with hexane, the color absorbed by silica gel is recovered by eluting with diethyl ether. Identification of colors is made by silica gel G thin-layer chromatography.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Glass plates of 20 x 20 cm 3. Applicator and board 4. Developing tank		
Materials and Reagents	1. Silica gel - G for TLC/HPTLC plate 2. Silica gel (column chromatography grade) 3. Hexane 4. Diethyl ether 5. Benzene 6. Acetic acid 7. Oil soluble colors		
Preparation of reagents	1. Solvent mixture of benzene, hexane and acetic acid in 60:40:1 (v/v). 2. Standard solutions of known oil soluble colors (0.1% solution in oil).		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Take about 5 mL of oil sample in a glass stoppered conical flask. 2. Add 25 mL of hexane followed by 10 g silica gel (column		

	chromatography grade) and 2 g anhydrous sodium sulphate. 3. Stir the mixture well and keep aside for 5 min. Decant off the solvent. 4. Add once again 25 mL of hexane and stir well and decant the solvent. Likewise add hexane 25 mL 3-4 times to the flask and draining out the solvent each time to remove almost all the oil leaving behind the silica gel in the flask. Thin Layer Chromatography 5. Elute the coloring matter absorbed by silica gel in the flask by shaking with diethyl ether 2 - 3 times using 20 mL each time. 6. Collect the diethyl ether extract in a beaker. 7. Evaporate the solvent on a hot water bath. 8. Spot the concentrated ether extract using capillary tube on an activated plate. 9. Develop the plate in a tank containing solvent mixture. 10. Remove the plate when the solvent layer has reached 12 to 15 cm height and dry at room temperature. 11. Heat the plate at 100 °C in an oven for 1 h and observe. 12. Natural colors like carotenes would fade away leaving oil soluble coal tar colors.
Inference (Qualitative Analysis)	Compare the spots with spots of known oil soluble colors spotted side by side and identify the color.
Reference	Manual Methods of Analysis for adulterants and contaminants in Foods ICMR (1990) page 16
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method for presence of beef fat in lard (Pork fat)		
Method No.	FSSAI 02.048:2021	**Revision No. & Date**	0.0
Scope	Fats are mixed to obtain commercial advantage depending on their availability.		
Caution	1. Potassium hydroxide: corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Material is extremely destructive to all body tissues. May be fatal if swallowed. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases.		
Principle	The presence of beef fat, tallows and similar fats as well hydrogenated and interesterified pork fat in lard is detected by determining difference between melting point of crystallized glycerides and the melting point of fatty acids derived from these glycerides. The value is large for pure pork fat and small for beef fat.		
Apparatus	General glassware and apparatus (Refer 2.0 at page no. 1)		
Materials and Reagents	1. Acetone 2. Potassium hydroxide 3. Ethyl alcohol 4. Hydrochloric acid		
Preparation of reagents	0.5N alcoholic Potassium hydroxide		
Sample Preparation	Refer 3.0 at page no. 2		
Method of Analysis	1. Weigh 5 g of melted and filtered lard into glass stoppered cylinder. 2. Add 20 mL warm acetone. Mix well, taking care that solution is clear above 30 °C. 3. Let stand 16-18 h at constant temperature of 30 °C. Fine mass of crystals not less than 3 mL should be found at the bottom of the cylinder. 4. If volume of crystals exceeds 3 mL, take smaller amounts of lard (3-4 g) for new test. If crystals obtained from 5 g lard are insufficient increase weight of lard and volume of acetone proportionately. 5. Decant supernatant acetone solution from crystallized glycerides. Add three 5 mL portions of warm acetone (30 – 35 °C) taking care not to breakup deposit in washing and decant first 2 portions. 6. Actively agitate third portion and by quick movement transfer crystals to small filter paper. 7. Using wash bottle wash crystals with 5 successive small portions of warm acetone. 8. Spread out paper and contents breaking up any large lumps and air dry at room temperature. 9. Thoroughly comminute mass and determine melting point (mp) of crystals in closed 1 mm tube.		

	10. Melting point is reached when fused substance becomes perfectly clear and transparent. 11. When melting point of glycerides is less than 63 °C presence of beef fat or other fat should be suspected. 12. Confirm presence of foreign fat by taking up melting point of fatty acids prepared from glycerides. 13. Transfer crystallized glycerides to 50 mL beaker, add 25 mL of approx 0.5 N alcoholic KOH and heat on steam bath until saponification is complete. 14. Pour solution into separator containing 200 mL water, acidify, add 75 mL ether shake and let stand. Drain aqueous acid layer and wash ether solution 3 times with water. 15. Transfer ether solution to dry 50 mL beaker, evaporate ether on steam bath and finally dry acids at 100 °C. 16. Let acids remain at room temperature for 2 h and determine melting point.
Inference (Qualitative Analysis)	If melting point of glycerides plus twice difference between melting point of glycerides and melting point of fatty acids is less than 73 °C lard is regarded as adulterated.
Reference	1. AOAC 17th edn, 2000, Official Method 920. 163 Fats (Foreign) containing tristearin in lard. 2. Pearsons Composition and Analysis of Foods 9th edn, page 611.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of residual hexane in oils and fats		
Method No.	FSSAI 02.049:2021	**Revision No. & Date**	0.0
Scope	The residual hexane content is the quantity of volatile hydrocarbons remaining in the fats and oils following processing involving the use of solvents.		
Caution	1. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 2. Heptane: n-Heptane can irritate the eyes, nose, and throat. Exposure can cause headache, lightheadedness, dizziness, lack of coordination and loss of consciousness. Loss of appetite and/or nausea may occur. n-Heptane is a flammable liquid and a dangerous fire hazard.		
Principle	The volatile hydrocarbons are desorbed by heating the sample at 80 °C in a closed vessel after addition of an internal standard. After determination of a calibration factor, hydrocarbons in the head space are determined by gas chromatography using packed or capillary columns. Results are expressed as hexane in mg/kilogram (mg/kg, or ppm). The method is applicable to the determination of 'free' volatile hydrocarbons expressed in terms of hexane remaining in animal and vegetable fats and oils after extraction with hydrocarbon-based solvents. It is suitable for determination of quantities of hexane between 10 and 1500 mg/kg in fats and oils.		
Apparatus / Instruments	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Gas Chromatograph having (a) Thermostatic column capable of maintaining the desired column temperature within ±1 °C, (b) Sample inlet system, separately thermostatted which can be maintained at a minimum temperature of 100 °C. If a capillary column is used, the inlet system must be capable of a 1/100 split injection. For serial analysis a headspace gas chromatograph with automatic sample injection and tempering bath is satisfactory (c) Flame ionization detector which can be separately thermostatted and maintained at a minimum of 100 °C 3. Recorder - If a recorder trace is to be used for calculating the composition of the samples analyzed, an electronic recorder of high precision is required or 4. Electronic Integrator (preferred) which permits rapid and accurate calculations. 5. Chromatographic Column – Either packed or capillary column with the following minimum requirements (a) Packed Column - stainless steel or glass, approx 2 m long and 1 / 8 inch internal diameter with acid washed and silanised diatomaceous earth,150 - 180 µ particle size (80 - 100 mesh Chromosorb WAW is suitable), stationery phase – squalene consisting of 10% of packing		

<table>
<tr><td></td><td>(b) Capillary column – glass or fused silica approx 30 m long and 0.3 mm internal diameter
Stationary phase – Methyl polysiloxane (film thickness 0. 2 μ)
6. Syringe – 1 μL, 10 μL, 1000 μL capacity, gas tight.
7. Septum vial -20 mL capacity
8. Septa and Aluminium caps suitable for septum vials together with crimping pliers. The septa must be resistant to oils and solvents (butyl rubber or red rubber is recommended.)
9. Tongs suitable for holding septum vials
10. Heating bath with clamps for holding septum vials, thermostatically regulated and capable of maintaining a temperature of 80 °C. For continuous operation glycerol is recommended as heating liquid
11. Shaking machine.</td></tr>
<tr><td>Materials and Reagents</td><td>1. Gases
(a) Carrier – Helium (preferred for better resolution) or Nitrogen 99.99 % pure, dried and containing a maximum of 10 mg O_2/kg
(b) Flame Ionization Detector – Hydrogen, minimum purity 99.95%, Air or Oxygen, dry, hydrocarbon free (less than 2 ppm hydrocarbon equivalent to CH_4)
2. Technical Hexane or light petroleum with a composition similar to that used in industrial extraction or n–hexane. For calibration, technical extraction hexane is preferred
3. n- Heptane (internal standard) analytical reagent grade
4. Vegetable Oil - solvent free, freshly refined and deodorized. The oil is to be used for calibration and should be of a similar nature as the sample. It should be free from extraction solvent (less than 0.01%).</td></tr>
<tr><td>Sample Preparation</td><td>Refer 3.0 at page no. 2.
It is essential that loss of solvent from the sample be prevented. The laboratory sample should be in a completely sealed condition and stored at 4 °C. Plastic containers should not be used. Sample analysis should be carried out immediately when the sample container is opened.</td></tr>
<tr><td>Method of Analysis</td><td>Column Chromatography
GC Operating Conditions
1. Carrier gas flow depends on the carrier gas and the type of column being used for analysis and should be optimized accordingly.
2. The flow of hydrogen and air or oxygen to the FID should be optimized according to the manufacturer’s recommendation. Injector and detector temperatures should be set at about 250 °C. The column should be maintained at 40 °C.
Procedure
3. Determination of the calibration factor - Weigh to the nearest 0.01 g, 5 g of solvent free vegetable oil (reagent 4) into each of the 7 septum vials. Seal each vial with a septum and cap. By means of a syringe add technical Hexane to 6 of the seven vials (the vial with no added solvent is the blank) according to the following table:
<table>
<tr><td>μL / 5 g</td><td>0.5</td><td>1</td><td>2</td><td>4</td><td>7</td><td>10</td></tr>
<tr><td>mg/1000 g</td><td>67</td><td>134</td><td>268</td><td>536</td><td>938</td><td>1340</td></tr>
</table></td></tr>
</table>

4. One vial remains without the addition of solvent.
5. If n–hexane is used for calibration the following table applies.

µL / 5 g	0.5	1	2	4	7	10
mg/1000 g	66	132	264	528	924	1320

6. Shake the 6 vials containing the solvent in the shaking machine vigorously for 1 h.
7. Using the syringe add 5 µL of internal standard (reagent 3, n-heptane) to each of the 7 vials.
8. Successively immerse the vials upto the neck in the heating bath set at 80 °C at intervals of approx 15 min. This time interval depends on the duration of the GC analysis, which is complete on the elution of the internal standard (n – heptane).
9. The samples must be placed in the heating unit at intervals such that each sample is tempered for exactly 60 min.
10. Warm the gas tight syringe to 60 °C. After tempering at 80 °C for exactly 60 min and without removing the vial from the heating bath, use the gas tight syringe and withdraw through the septum 1000 µL (1 mL) of the head space above the oil.
11. Inject immediately into the gas chromatograph. For each of the vial containing added solvent a calibration factor F may be determined by the formula.

$$F = \frac{C_s \times A_1}{(A_H - A_B - A_1) \times C_1}$$

Where,

A_H = Total peak area of solvent hydrocarbons including the area of internal standard present in the spiked oil. For identification purposes a typical chromatogram of solvent composition should be obtained. Hydrocarbons which usually make up the technical hexane are 2-Methyl pentane, 3-Methyl pentane. Methyl cyclopentane, cyclohexane, etc. Do not include peaks due to oxidation products which may be present in significant amounts

A_B = Peak area of the solvent hydrocarbons present in the oil to which solvent has not been added (blank) less the peak area of the internal standard

A_1 = Peak area corresponding to the internal standard in the spiked samples

C_1 = Quantity of the internal standard added expressed in mg/kg of the oil

C_S = Quantity of technical hexane added to the oil present in the vial expressed in mg/kg of the oil

Express the results to the third decimal place.

12. Calibration factors of the six standards should be approximately the same. The mean calibration factor should be 0.45 if n–heptane is used and 0.57 if cyclohexane is used.
13. The factor (F) so evaluated can be used for determining vial

	quantities of hexane less than 60 mg/kg. If the value of F found for the vial containing 0.5 µL of hexane is significantly below the mean value, this deviation is probably due to difficulty in introducing exactly 0.5 µL and this determination must be either eliminated or repeated. 14. For quantities of hexane between 10 and 20 mg/kg it is better to prepare calibration standards by adding 2 µL of internal standard instead of 0.5 µL. **Sample Analysis** 15. Weigh to the nearest 0.01 g, 5 g of the test sample into a septum vial as quickly as possible and close immediately with a septum and cap. 16. Using a syringe add through the septum exactly 5 µL of the internal standard. Shake vigorously by hand for about 1 min and then immerse the vial upto the neck in the heating bath at 80 °C for exactly 60 min. 17. Warm the gas tight syringe to 60 °C. After tempering at 80 °C for exactly 60 min use the gas tight syringe and take from the vial without removing it from the bath 1000 µL (1 mL) of the head space above the sample. 18. Immediately inject into the gas chromatograph. Carry out two determinations in rapid succession on each sample
Calculation with units of expression	The residual solvent expressed in mg / kg (ppm) is given by the formula: $$W = \frac{(A_H - A_1) \times F \times C_1}{A_1}$$ Where, A_H = Total peak area of solvent hydrocarbons including the area of internal standard. Hydrocarbons which usually make up the technical solvents are 2 methyl pentane, 3 methyl pentane, methyl cyclopentane, cyclohexane etc. Do not include peaks due to the oxidation products. Some of these products may be present in significant amount. A_1 = Peak area corresponding to internal standard in the sample C_1 = Quantity of the internal standard added in mg/kg Note: - For an addition of 5 µL of heptane / 5 g of sample C1 = 680 mg/kg and C1 = 750 mg/kg if cyclohexane is used F = Calibration factor obtained in procedure Report the final result as a mean of two determinations.
Reference	AOCS 6thedn, 2012, Official Method Ca 3b. – 87
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for determination of trans fatty acids in hydrogenated vegetable oil**		
Method No.	FSSAI 02.050:2021	**Revision No. & Date**	0.0
Scope	During the partial dehydrogenation of vegetable oils (e.g., in the manufacture of margarine), the *cis*-fatty acids are isomerized to the *trans*-fatty acid forms. The "hydrogenated" margarines contain 15%–40% *trans*-fatty acids. The consumption of *trans*-fatty acids increases the risk of coronary heart disease by elevating atherogenic low-density lipoprotein (LDL) cholesterol and lowering antiatherogenic (cardioprotective) high-density lipoprotein (HDL) cholesterol		
Caution	1. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 2. Chloroform: Chloroform is irritating to eyes, respiratory system and skin. It poses danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Over pressurized containers of chloroform are potentially explosive. Wear nitrile gloves, lab coat, and safety glasses. 3. Boron trifluoride: Boron trifluoride is extremely corrosive. Caution is advised. Signs and Symptoms of Acute Boron Trifluoride Exposure: Acute inhalation exposure of boron trifluoride may result in sneezing, hoarseness, choking, laryngitis, and respiratory tract irritation. 4. Toluene: Toluene is a highly flammable liquid and it can cause mild damage to the skin and the eyes. However, the most-common hazard associated with this chemical is inhalation. Products containing toluene can produce dangerous fumes which can cause nausea, headaches, unconsciousness, and even death if inhaled. 5. Hexane: Exposure to hexane is most likely to occur in the workplace. It is recommended that you wear protective gloves, safety goggles, protective clothing and breathing protection when working with the chemical. Do not smoke, drink, or eat when exposed to hexane. 6. Sodium sulphate: Skin-May cause skin irritation. May cause an allergic reaction in certain individuals. Ingestion-May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May be harmful if swallowed.		
Principle	Triglyceride, triundecanoin (C11:0), is added as internal standard. Fat is extracted in to ether, then methylated to fatty acid methyl esters (FAMEs) using BF_3 in methanol. FAMEs are quantitatively measured by capillary gas chromatography (GC) against C 11:0 internal standard. Total transfat is calculated as sum of individual trans fatty acids expressed as triglyceride equivalents.		

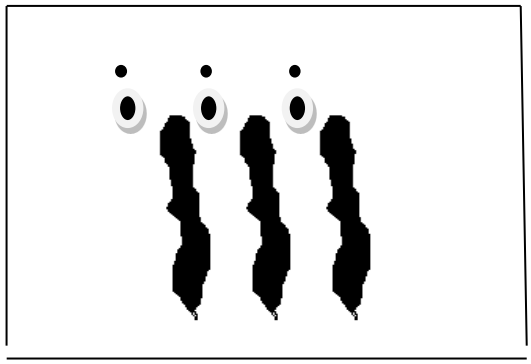
Apparatus / Instruments	General glassware and apparatus (Refer 2.0 at page no. 1). 1. Gas chromatograph (GC)-Equipped with hydrogen flame ionization detector, capillary column, split mode injector, oven temperature programming sufficient to implement a hold ramp hold sequence. Operating conditions: temperature (°C): injector, 225; detector, 285; initial temp, 100 (hold 4 min); ramp, 3°C/min; final temperature 240; hold 15 min; carrier gas, helium; flow rate, 0.75 mL/ min; linear velocity, 18 cm/s; split ratio, 200:1. 2. Capillary Column — Separating the FAME pair of adjacent peaks of C18:3 and C20:1 and the FAME trio of adjacent peaks of C22:1, C20:3, and C20:4 with a resolution of 1.0 or greater. SP2560 100 m × 0.25 mm with 0.20 µm film is suitable. 3. Water Bath — With nitrogen stream supply, maintaining 40±5 °C. 4. Gravity Convection Oven — Maintaining 100± 2 °C 5. Three Dram Vials — About 11 mL 6. Teflon/Silicone Septa — To fit vials
Materials and Reagents	1. Chloroform 2. Diethyl ether 3. Toluene Nanograde 4. Sodium sulfate — Anhydrous 5. Boron trifluoride 6. Internal Standard — C11:0-triundecanoin 7. Mixed FAMEs Standard Solution(CRM 47885),CRM 46903 8. Individual FAME Standard Solutions 9. Hexane
Preparation of reagents	1) Boron trifluoride Reagent: 7% BF_3 (w/w) in methanol, made from commercially available 14% BF_3 solution. Prepare in the hood. 2) Triglyceride Internal Standard Solution: C11:0-triundecanoin; 5.00 mg/mL in Chloroform. Accurately weigh 2.50 g C11:0-triundecanoin into 500 mL volumetric flask. Add ca 400 mL Chloroform and mix until dissolved. Dilute to volume with Chloroform. Invert flask at least 10 additional times. Triglyceride internal standard solution is stable up to 1 month when stored in refrigerator (2-8 °C). 3) Mixed FAMEs Standard Solution: Reference mixture containing series of FAMEs, including C18:1 cis and trans (available commercially, or equivalent). To prepare mixed FAMEs standard solution break top of glass vial, open and carefully transfer contents to 3-dram glass vial. Wash original vial with hexane to ensure complete transfer and add washings to 3-dram glass vial. Dilute to ca 3 mL with hexane. 4) Individual FAME Standard Solutions: Standard FAMEs solutions of several *cis* and *trans* fatty acids are available commercially and the required trans fatty acid standards can be used. Prepare individual FAME standard solutions as follows: Break top of glass vial open and carefully transfer contents to 3-dram glass vial. Wash original vial with hexane to ensure complete transfer and add washings to 3-

	dram glass vial. Add 1.0 mL C11:0 FAME standard solution. Dilute to total volume of ca 3.0 mL with hexane. Individual FAME standard solutions are stable up to 1 week when stored in refrigerator (2 – 8 °C).
Sample Preparation	Refer 3.0 at page no. 2
Method of Analysis	Methylation to FAMEs 1. Accurately weigh ca 100-200 mg of vanaspati or hydrogenated fat. 2. Dissolve in 2-3 mL chloroform and 2-3 mL diethyl ether. 3. Transfer mixture to 3-dram glass vial and then evaporate to dryness in 40 °C water bath under nitrogen stream. 4. Add 2.0 mL 7% BF3 reagent and 1.0 mL toluene. Seal vial with screw cap top with Teflon/silicone septum. 5. Heat vial in an oven for 45 min at 100 °C. Gently shake vial ca every 10 min. *NOTE — Evaporation of liquid from vials indicates inadequate seals: if this occurs, discard solution and repeat the entire procedure.* 6. Allow vial to cool to room temperature (20-25 °C). 7. Add 5.0 mL water, 1.0 mL, hexane, and ca 1.0 g anhydrous sodium sulphate. 8. Cap vial and shake for 1min. Allow layers to separate and then carefully transfer top layer to another vial containing ca 1.0 g anhydrous sodium sulphate. *NOTE —Top layer contains FAMEs including FAME of triglyceride internal standard solution.* Column Chromatography GC of FAMEs 9. Relative retention time (vs FAME of triglyceride internal standard solution) and response factors of individual FAMEs can be obtained by GC analysis of individual FAMEs standard solution and mixed FAME standard solution. 10. Inject ca 2 µL each of individual FAMEs standard solutions and 2 µL of mixed FAMEs standard solution. 11. Use mixed FAMEs standard solution to optimize chromatographic response before injecting any test solutions. 12. After all chromatographic conditions have been optimized, inject test solutions. *NOTE — With matrices of unknown composition, it may be necessary to analyze test portion without addition of internal standard to ensure against interferences. Should interfering peak be found, the area of C11 internal standard peak must be corrected before performing calculations. Use 2.0 mL chloroform instead of internal standard solution.*
Calculation with units of expression	Calculate retention times for each FAME in individual FAMEs standard solutions (D-3.13.3), by subtracting retention time of C11:0 peak from

	retention time of fatty acid peak. Use these retention times to identify FAMEs in mixed FAMEs standard solution. Use additional FAME solutions (from the same supplier) when necessary for complete FAME identity verification. Calculate percent of trans fat in test sample [w/w; expressed as sum of only trans fatty acids (C14:1,Trans Myristelaidic + C16:1, Trans Palmitelaidic + C18:1,Trans 6 Petroselenic + C18:1,TransElaidic + C18:1,Trans 11 Vaccenic + C18:2, Trans Linolelaidic+ C18:2, Trans 9-Linolelaidic+C18:2, Trans 12-Linolelaidic + C18:3, Trans Linolenic + C20:1,Eicosenic Trans 11)] as follows: $$\text{Transfat, (\%)} = \frac{\sum transfat\ W_i}{\sum W_{test\ portion}} \times 100$$ Wi= weight of individual trans FAME in mixed FAMEs standard solution W_{test}=weight of individual trans FAME in test sample *NOTE —Test samples containing hydrogenated fat will yield complicated chromatograms due to large number of isomers formed during hydrogenation process. One general indication of hydrogenation is presence of C18:1transpeak(s). Transpeaks elute prior to cis, therefore, include all peaks between C18:1cis and C18:2 cis, cis in calculation of C18:2 peak area. Often C18:1 trans "peak" consists of broad series of peaks [due to positional isomers from hydrogenation]; include all of these in C18:1 trans peak area.*
Reference	1. AOAC Method 965.34 in *Official Methods of Analysis* of the *Association of Official Analytical Chemists* (Helrich, K. ed), 1994. Arlington, Virginia. 2. AOAC Method 965.34 in *Official Methods of Analysis* of the *Association of Official Analytical Chemists* (Helrich, K. ed), 1994. Arlington, Virginia. 3. Official Method Cd-14-61 in Official Methods and Recommended Practices of the Americal Oil Chemist's Society (Firestone, D., ed) 1993 AOCS Press Champaign, Illinois
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total polar compounds in edible oils and fats		
Method No.	FSSAI 02.051:2021	**Revision No. & Date**	0.0
Scope	The method determines the extent to which fats and oils deteriorate when used for frying. Note: Polar components include polar substances such as monoglycerides, diglycerides, free fatty acids that occur in unused fats, as well as polar transformation products formed during frying of foodstuffs and/or during heating. Nonpolar components are mostly unaltered triglycerides.		
Caution	1. Diethyl ether: Diethyl ether is a volatile chemical that can easily catch fire or even explode. This chemical also poses an inhalation hazard, and can cause irritation of the eyes and skin. Due to these hazards, it's important to use caution whenever handling diethyl ether or being in its general vicinity. 2. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness.		
Principle	These fats and oils can be separated by the process of Silica Gel based column chromatography into polar and non-polar components. These components of fats can be determined by column chromatography under specified conditions.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 1). 2. Column - Glass, 2.1 cm id ×45 cm with Teflon Stopcock and ground-glass joint. 3. TLC plates – Pre coated silica gel (without fluorescence indicator), 20 × 20 cm, layer thickness = 0.25 mm.		
Materials and Reagents	1. Oils / Fats 2. Silica gel 60 (Adsorbent) - particle size 0.063-0.200 mm (70-230 mesh ASTM) 3. Petroleum ether (bp 40°- 60 °C). 4. Diethyl ether 5. Sea-sand - Analytical reagent grade 6. Molybdophosphoric acid 7. Ethyl alcohol		
Preparation of reagents	1. Silica gel 60 (Adsorbent) - particle size 0.063-0.200 mm (70-230 mesh ASTM), adjust to H_2O content of 5% as follow: Dry silica gel≥4 h in porcelain dish in 160 °C oven; cool in desiccator to room temperature. Adjust H_2O content to 5%, e.g., weigh 152gsilica gel and 8 g H_2O in 500 mL round-bottom flask with ground-glass stopped and mechanically shake 1 h. 2. Petroleum ether (b.p.40-60 °C) - ether (87+13) [Eluting solvent mixture]. 3. Sea-sand - Analytical reagent grade; purified by acid and calcined. 4. Spray reagent- Molybdophosphoric acid, 10% in alcohol.		

Sample Preparation	1. Semi liquid and solid fats are warmed to temperature slightly above melting point and mix thoroughly such as to avoid overheating. 2. Visible impurities are removed by filtration. Hydrophobic filter are to be used, if water is present. Refer 3.0 at page no. 2.
Method of Analysis	Refer below Column chromatography and Thin layer chromatography **Column Chromatography** Preparation of Column 1. Column is to be prepared using 30 mL (approx.) of petroleum ether-ether (87+13). Also place wad of cotton wool in bottom of column and remove air by pressing with glass rod. 2. Prepare slurry of 25 g silica gel and approx 80 mL petroleum ether-ether (87+13) in 100 mL glass beaker. Pour the slurry into column using 8 cm glass funnel. Beaker, funnel and sides of column are to be rinsed with same solvent. Open stopcock and drain solvent to 10 cm above silica gel. Silica gel is leveled by tapping the column. 3. Approx 4 g of sea-sand is added through funnel into column. Solvent is drained to sand layer. Chromatography 4. Only nonpolar fraction is used to determine polar components by difference. However, if separation is controlled by TLC, both polar and nonpolar fractions are required. 5. Separation may also be controlled by checking recovery of analytes. But for products containing substantial amounts of polar material, recovery may be incomplete because small amounts of highly polar material, generally 1-2%, are not eluted under conditions specified. 6. 2.5 ± 0.1 g (to 0.001 g) test portion is accurately weighed into 50 mL volumetric flask and dissolved in approximately 20 mL petroleum ether-ether (87+13) while warming slightly. 7. Let it cool to room temperature and dilute to volume with same solvent. 8. 20 mL aliquot is transferred to column using volumetric pipet, without disturbing surface. 9. Two 250 mL round-bottom flasks are dried in 103° ± 2 °C oven, cool to room temperature, and accurately weigh to 0.001 g. 10. One flask is placed under column, stopcock is opened, and solution is drained to level of sand layer. 11. Nonpolar components are eluted with 150 mL petroleum ether-ether (87+13) contained in 250 mL dropping funnel. 12. Flow rate is adjusted such that 150 mL passes through column within 60-70 min. 13. After elution, wash any substance adhering to outlet of column into round-bottom flask with petroleum ether-ether (87 + 13). 14. In same manner, polar components are eluted into second 250 mL round-bottom flask with 150 mL ether. Silica gel is discarded. 15. Solvent is removed from each fraction with a rotary evaporator and 560 °C water bath or with N_2 Stream in 250 mL flask on steam bath.

	16. Avoid losses due to foaming. If rotary evaporator is used, shortly before end of evaporation, introduce N_2 into system. Cool residue to ambient temperature and introduce N_2 into flask. Weigh flasks. Check of Column Chromatography Efficiency by Thin-Layer Chromatography **Thin Layer Chromatography** 17. Dilute polar and nonpolar fraction (1+9) in $CHCI_3$. 18. Apply 2µL spots using capillary dispensing pipet. 19. Develop plate with petroleum ether-ether-CH_3COOH (70+30+2) in tank lined with filter paper for approximately 35 min (ca 17 cm). Remove plate and let solvent evaporate. 20. Spray plate with 10% molybdophosphoric acid. After evaporation of alcohol, heat plate in 120°-130 °C drying oven. Fraction 1 (nonpolar) should be free of polar substances (see Figure 1).  1 2 1 2 1 2 FRACTION Figure1. Evaluation of efficiency of fractionation by TLC separation of polar and nonpolar fraction: Fraction 1 contains nonpolar components, and Fraction 2 contains polar components.
Calculation with units of expression	Calculate polar components, as percent (w/v) with formula: Polar components, % $= \frac{E-A}{E} \times 100$ Where A = nonpolar fraction (in g); E= test portion (in g) in 20 mL aliquot (ca 1 g). Report result to one decimal place.
Reference	AOAC Official Method 982.27
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Spectrophotometric method for determination of gamma Oryzanol in Rice bran oil and other vegetable oils		
Method No.	FSSAI 02.052:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for the quantitative determination of oryzanol in crude and refined rice bran oils. The method can also be used for the detection of adulteration of rice bran oil in other crude and refined oils. **Note:** For total oryzanol content, UV Spectrophotometer method may be used and in case of label claim for gamma oryzanol, HPLC method shall be used.		
Caution	n-Heptane: n-Heptane can irritate the eyes, nose and throat. Exposure can cause headache, lightheadedness, dizziness, lack of coordination and loss of consciousness. Loss of appetite and/or nausea may occur.		
Principle	Absorbance values of diluted oil is measured and percentage of oryzanol determined.		
Apparatus	1. General glassware and apparatus (Refer 2.0 at page no. 1) 2. Spectrophotometer - for measuring extinction in the ultraviolet between 310 and 320 nm. 3. Rectangular quartz cuvettes - having an optical light path of 1 cm. 4. Volumetric flask – 25 mL 5. Filter paper - Whatman no.2, or equivalent.		
Materials and Reagents	1. Oils and fats 2. n-Heptane - Spectrophotometrically pure		
Sample Preparation	Filter the oil sample through filter paper at ambient temperature.		
Method of Analysis	1. Before using, the spectrophotometer should be properly adjusted to a zero absorbance filling both the sample cuvette and the reference cuvette with n-Heptane. 2. Weigh accurately about 0.02 g of the sample so prepared into a 25 mL volumetric flask, make up to the mark with n-Heptane. 3. Fill a cuvette with the solution obtained and measure the extinction at the wavelength of maximum absorption near 315 nm, using the same solvent as a reference. 4. The extinction values recorded must lie within the range 0.3-0.6. If not, the measurements must be repeated using more concentrated or more diluted solutions as appropriate.		
Calculation with units of expression	Calculate gamma oryzanol content as follows: Gamma oryzanol content (in %) = 25 × (1/W) × A × (1/E) Where W = mass of sample A = extinction (absorbance) of the solution. E = specific extinction $E_{1\ cm}^{1\%}$= 359		
Reference	CODEX Alimentarius Commission – CODEX STAN 210-1999		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**HPLC method for determination of gamma oryzanol content in rice bran oil and other vegetable oils**		
Method No.	FSSAI 02.053:2024	**Revision No. & Date**	0.0
Scope	Method is useful to determine gamma oryzanol content in rice bran oil as well as other vegetable oils. Gamma oryzanol / Oryzanol is mixture of four compounds viz., cycloartenyl ferulate (CycloFer), 24-methylene cycloartanyl ferulate (24-MCFer), campesteryl ferulate (CampFer), and β-sitosteryl ferulate (β-SitFer). **Note 1:** This method is also useful to determine tocopherols and sterols present in oils. However, details are not provided here. **Note 2:** For total oryzanol content, UV Spectrophotometer method may be used and in case of label claim for gamma oryzanol, HPLC method shall be used.		
Caution	1. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. 2. Dichloromethane: Higher levels of dichloromethane inhalation can lead to headache, mental confusion, nausea, vomiting, dizziness and fatigue. Skin Exposure - Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns.		
Principle	Fats and Oils are diluted, filtered and analysed by RP-HPLC using PFP column and diode array detector@328nm for total oryzanol content. **Notes:** 1. Method validation showed linearity of calibration curves (α=0.05). 2. RSD of intra-day, inter-day and inter-laboratory precision were less than 4.88%. **3.** The limit of detections (LODs) and limit of quantifications (LOQs) were low (0.009–2.166 µg/g) with recoveries around 96.0–102.9%.		
Apparatus/Instruments	1. General standard glass ware. 2. HPLC connected to a diode array detector. 3. Pentafluorophenyl propyl (PFP) core-shell column (4.6×250 mm, 5 µm; It is alternative to the widely used C18 and C8 phases. 4. 0.45 µm syringe nylon filter. 5. HPLC syringe. **Notes:** (i) Any HPLC system with a diode array detector and PFP column, which can provide proper resolution of the target compounds). (ii)The PFP core-shell column (4.6×250 mm, 5 µm) is a robust core phase that reduces method development time with its dynamic and		

	responsive chemical functionality. With five retention mechanisms and five separation modes, this column is an alternative to the widely used C18 and C8 phases.
Materials and Reagents	1. Rice bran oil 2. Vegetable oils 3. Individual standards of γ-oryzanols (CycloFer, 24-MCFer, CampFer, and β-SitFer). (or) a Mixture of standard γ-oryzanols (CycloFer, 24-MCFer, CampFer, and β-SitFer at a known ratio). 4. Methanol – HPLC grade. 5. Water – HPLC grade. **6.** Dichloromethane – HPLC Grade.
Preparation of Reagents	Degass all the HPLC solvents before use.
Sample Preparation	Dilute the oils (0.5 g) with dichloromethane and adjust the volume to 1.00 mL. Filter the resulting solution through a 0.45 μm syringe nylon filter and analyze.
Method of analysis	**HPLC analysis** 1. Mobile Phases - Component A: Methanol; Component B: Water 2. The gradient elution was 90% A (0–13 min), linearly changed to 95% A (13–14 min), linearly changed to 85% A (14–17 min), linearly changed to 95% A (17–22 min), and then held at 95% A (22–30 min). 3. The flow rate is 1.0 mL min−1 4. The column temperature is 30 °C. 5. Sample Volume – 5 mL/each injection. 6. Determine LOD and LOQ using standards. 7. Signal-to-noise (S/N) ratio was determined by comparing signals from sample with known low concentrations of compound with noise of blank samples. S/N ratios of 3 and 10 were for LOD and LOQ, respectively. 8. Prepare the standard curves using known concentrations (five or eight). Concentration vs peak area is standard curve for respective compound. 9. Inject the oil samples and analyse. Triplicate injections are preferred. 10. Note the peak areas. **Notes:** (i) The elution order (retention times) derived from the PFP column is CycloFer (24.52 min), 24-MCFer (25.63 min), CampFer (26.62 min), and β-SitFer (27.50 min). These may vary depending on the column phase, mobile phase and flow rate. (ii) LODs for CycloFer, 24-MCFer, CampFer and β-SitFer are 0.215, 0.218, 0.216 and 0.714 mg/mL respectively. LOQs for CycloFer, 24-MCFer, CampFer and β-SitFer are 0.651, 0.647, 0.632 and 2.166 mg/mL respectively.
Calculation with units of expression	Calculate the quantities of each oryzanol components using respective peak areas and standard curves.

	Total oryzanol quantity is determined by adding /combining all the quantities of oryzanol components. Express the Total oryzanol quantity for 100 g of oil.
Reference	Simultaneous determination of tocols, γ-oryzanols, phytosterols, squalene, cholecalciferol and phylloquinone in rice bran and vegetable oil samples by Piramon Pokkanta, Phumon Sookwong, Manatchanok Tanang, Saranya Setchaiyan,Pittayaporn Boontakham, Sugunya Mahatheeranont, Food Chemistry 271 (2019) 630–638.
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RAPID ANALYTICAL FOOD TESTING (RAFT) KIT/ EQUIPMENT

Alternate Rapid kits/equipments may be used to get quick results for screening and surveillance purposes, provided the kit/equipment is approved by FSSA(I). Details of the rapid food testing kit/ equipment approved by FSSA(I) are available at https://www.fssai.gov.in/cms/raft.php

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