Foreword

This Bangladesh Standard was adopted by the Bangladesh Standards and Testing Institution on ……., after the recommendation by the Sectional Committee for the Oilseeds and their Products had been approved by the Agricultural and Food Products Divisional Committee.

Palm olein is the liquid fraction obtained from the controlled fractionation of palm oil (*Elaeis guineensis*), where the solid glycerides (stearin) crystallize and are separated, leaving the liquid fraction (olein). This process enhances clarity and ensures the product remains liquid at ambient temperatures typical of warm climates. Refined palm olein has a balanced fatty acid profile dominated by palmitic (SFA), oleic (MUFA), and linoleic (PUFA) acids. These fatty acids determine the oxidative stability, melting behaviour, and nutritional properties of palm olein.

Edible palm olein is one of the most widely consumed frying and cooking oils in Bangladesh due to its stable performance, affordability, and suitability for fortification. As fortified edible oils continue to serve as a major vehicle for vitamin A delivery at the population level, palm olein is regulated under a strengthened framework of quality and safety. This standard specifies the essential requirements for fortified palm olein to safeguard public health, protect consumers, and support fair practices in trade.

This standard 'BDS 1774 Fortified Palm Olein' was first published in 2006 and subsequently amended in 2014 and 2021. This Bangladesh Standard is the first revision of BDS 1770. Major modifications in this version are as follows:

i) clauses for 'Normative References' 'Fatty Acids Profiling', 'Legal Requirements', 'Hygienic Requirements', 'Pesticide Residues', 'Permitted Food Additives' and 'Compliance' have been incorporated;
ii) 'Acid value' parameter has been removed and limits for ''Insoluble impurities', 'Free fatty acid (as palmitic)' and 'Relative density' have been added;
iii) new parameters with limit like 'Total carotenoids (as ß carotene)', 'Trans fatty acids', 'Soap content', 'Flash point', 'n-Hexane residues' and '3-MCPD Esters and Glycidyl Esters (GE)' have been included;
iv) limits for different heavy metals have been added; and
v) requirements for labeling have been modified according to the current practice.

The Technical Committee responsible for the preparation of this standard has taken into consideration the views of the members of the committee, local refiners, producers, consumers, nutrition experts and technologists, and has related the standard to domestic manufacturing and commercial practices.

In the formulation of this standard, considerable assistance has been derived from the following publications, which are acknowledged with thanks:

i) *CXS 210-1999 Standard for Named Vegetable Oils*, last revised in 2019 and amended in 2024, Codex Alimentarius Commission; and

ii) MS 816:2025 Palm olien – Specification
Department of Standard Malaysia

This standard is subject to periodic review and amendment, if necessary, in order to keep pace with technological advancements and market developments. Suggestions for improvement received from stakeholders will be recorded and placed before the committee for consideration in due course.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with BDS 103. The number of significant figures retained in the rounded-off value shall be the same as that of the specified value in the standard.

This standard BDS 1774:YYYY Fortified Palm Olein (1st Rev.) cancels and replaces BDS 1774:20006, Fortified Palm Olein, Amendment-2:2021 which has been technically revised.

Bangladesh Standard
Specification for
Fortified Palm Olein
(*First Revision*)

1. Scope

1.1 This standard prescribes requirements and methods of sampling and test for fortified palm olein for edible purposes.

2. Normative References

2.1 The relevant standards listed in Annex-A are necessary adjuncts to this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

3. Terminology

3.1 For the purpose of this standard, the following definitions shall apply.

3.2 Crude palm oil – Crude palm oil is the oil derived by mechanical expression from the fleshy pulp (mesocarp) of the fruit of *Elaeis guineensis.*

3.2 Crude palm olein – The low-melting (liquid) fraction obtained by a one-stage fractionation of crude palm oil.

3.3 Fortified edible palm olein – The liquid fraction obtained by the fractionation of refined palm oil or crude palm oil that has been neutralized with alkali, bleached with food-grade bleaching earth and/or activated carbon, and deodorized with steam, without the use of other chemical agents, and fortified with Vitamin A. Alternatively, deacidification, bleaching and deodorization may be carried out by physical means.

4. Requirements

4.1 Description – The material shall be the liquid fraction derived by fractionation of palm oil obtained from the fleshy mesocarp of the fruits of oil palm (*Elaeis guineensis*) tree by the process of expression.

4.2 The material shall be clear on melting and free from adulterants, sediment, suspended and other foreign matter, separated water, and added colouring or flavouring substances and shall have acceptable taste and odour.

4.2.1 The clarity of the material shall be judged by the absence of turbidity after keeping the filtered sample at 40 ºC for 24 hours.

4.3 Admixture with other oils – The materials shall be free from admixture with mineral or other oils vegetable or animal origin when tested according to the prescribed in cl. 17 of BDS 1584 (Part-II).

4.4 Fatty Acids Profiling – The fatty acid profile should be determined by Gas Liquid Chromatography. Ranges of fatty acids are as given in Annex B. Samples falling within the appropriate ranges specified in Table B.1 are in compliance with this standard.

4.5 Hygiene – During processing, handling, storage and transportation, effective measures must be taken to prevent cross contamination with chemicals, microbial or physical contaminants.

4.5.1 The product shall be processed and packed under strict hygienic conditions in premises maintained in accordance with BDS 822.

4.6 Pesticide residues – The product covered by this standard shall comply with the maximum residue limits for pesticide established by the Codex Alimentarius Commission.

4.7 Specific requirement – The product shall also comply with the requirements given in Table-1.

Table – 1 Requirements for Fortified Palm Olein

Sl. No.	Characteristics	Requirements	Method of Test
(1)	(2)	(3)	(4)
i.	Moisture, percent by weight, *Max.*	0.1	BDS ISO 662
ii.	Insoluble impurities, percent by mass, *Max.*	0.05	BDS ISO 663
iii.	Colour in a 5.25 inch cell on the Lovibond scale expressed as Y+5R a) Minimum b) Maximum	a) R=1.5 Y=15 b) R=3 Y=30	BDS ISO 15305
iv.	Slip Melting point, ºC *Max.*	24	BDS ISO 6321
v.	Free fatty acid (as palmitic), percent by mass, *Max.*	0.25	BDS ISO 660
vi.	Refractive index at 40 ºC	1.4550 - 1.4610	BDS ISO 6320
vii.	Relative density at 27 ºC	0.910-0.915	BDS ISO 6883
viii.	Saponification value (as KOH) mg/g	195 - 205	BDS ISO 3657
ix.	Iodine value (Wijs)	56 – 61	BDS ISO 3961
x.	Peroxide value, meq/kg, *Max.*	10	BDS ISO 3960
xi.	Unsaponifiable matter, percent by mass, *Max.*	1.2	BDS ISO 18609/ BDS ISO 3596
xii.	Total carotenoids (as ß carotene), mg/kg	500-1200	ISO 17932
xiii.	Vitamin A (as Retinyl palmitate or retinyl acetate), mg/kg	15-30	Annex C
xiv.	Trans Fatty Acids, percent by weight, *Max.*	2	BDS ISO 12966-4
xv.	Flash point (Pensky-Martens), closed, ºC, *Min.*	250	BDS ISO 15267
xvi.	Soap content, percent by mass, *Max.*	0.005	BDS ISO 10539
xvii.	n-Hexane, mg/kg, *Max.*	1.00	Annex D

4.8 The product shall not contain any of the contaminants in excess of the quantities prescribed in Table-2.

Table 2 Limits for Contaminants

Sl. No.	Characteristics	Limit	Method of Test
(1)	(2)	(3)	(4)
i.	Arsenic (As), mg/kg, *Max.*	0.1	AOAC 986.15
ii.	Lead (Pb), mg/kg, *Max.*	0.08	AOAC 994.02
iii.	Cadmium (Cd), mg/kg, *Max.*	0.1	AOAC 2013:06
iv.	Mercury (total), mg/kg, *Max.*	0.25	AOAC 971.21
v.	Copper (Cu), mg/kg, *Max.*	0.1	AOAC 990.05
vi.	Iron (Fe), mg/kg, *Max.*	1.5	AOAC 990.05
vii.	Total Aflatoxin, µg/kg, *Max.*	5.0	BDS ISO 16050
viii.	3-MCPD Esters and (GE), mg/kg, *Max.*	2.5	BDS ISO 18363-4
ix.	Glycidyl Esters, mg/kg, *Max.*	1	BDS ISO 18363-4

4.9 Legal Requirement – The product shall in all other aspects comply with the requirements of the legislations enforced in the country.

5. Permitted Food Additives

5.1 Addition of the following food additives to the material not exceeding the prescribed levels are permitted:

INS No.	Antioxidants	Maximum Use level
304	Ascorbyl palmitate	500 mg/kg (singly or in combination)
305	Ascorbyl stearate	
307a	Tocopherol, d-*alpha*	300 mg/kg (singly or in combination)
307b	Tocopherol concentrate, mixed	
307c	Tocopherol, dl-*alpha*	
310	Propyl gallate	100 mg/kg
319	Trtiary butyl hydroquinone(TBHQ)	120 mg/kg
320	Butylated hydroxyanisole (BHA)	175 mg/kg
321	Butylated hydroxytolouine (BHT)	75 mg/kg
any combination of gallates, BHA, or TBHQ not to exceed 200 mg/kg within individual limits		
389	Dilauryl thiodiproprionate	200 mg/kg
330	Citric acid	GMP
331(i)	Sodium dihydrogen citrate	GMP
331(iii)	Trisodium citrate	GMP
384	Isopropyl citrates	100 mg/kg (Singly or in combination)
472c	Citric and fatty acid esters of glycerol	
Antifoaming agents (oils for deepfrying)		
900a	Polydimethylsiloxane	10 mg/kg

6. Packing and Marking

6.1 **Packing** – The material shall be packaged in containers made from food grade packaging material and sealed in a manner that will safeguard the hygienic, nutritional and organoleptic properties of the product throughout the shelf life of the product.

6.2 **Marking** – Each package shall be suitably labeled so as to give the following information:

a) Name of the product with brand name if any;
b) Name and address of the manufacturer, packer, distributor, importer, exporter or vendor, as appropriate;
c) Batch or code number;
d) Net content in millilitre/litre;
e) Date of manufacture and expiry;
f) Maximum Retail Price (MRP);
g) List of additives in descending order of proportion, if used;
h) Vitamin A Fortification shall be labeled as Vitamin A (Retinyl palmitate)/Vitamin A (Retinyl acetate); and
i) Any other requirements as specified under the Packaged Commodities Rules, 2021 (Amendment-2025)' of BSTI.

6.2.1 Each package may also be marked with the BSTI Certification Mark.

NOTE – The use of BSTI Certification Mark is governed by the provisions of Bangladesh Standards and Testing Institution Act, 2018 and the Rules and Regulations made thereunder. Details of conditions, under which a license for the use of BSTI Certification Mark may be granted to manufacturers or processors, may be obtained from the Bangladesh Standards and Testing Institution.

6.2.2 **Fortification logo –** "In addition to the BSTI Certification Mark, the 'Vitamin A Fortification Logo' must be conspicuously displayed on the label, container, packet, or bottle of the product in accordance with the provisions of 'The Law of Vitamin 'A' Fortification in Edible Oil, 2013".

7. Sampling

7.1 Representative samples of the material shall be drawn and conformity of the material to the requirements of the specification shall be determined according to the procedure given in BDS ISO 5555.

8. Tests

8.1 Test shall be carried out as prescribed method in col. 4 of Table 1, Table 2 and Annex B.

8.2 Quality of Reagents – Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (BDS 833) shall be used where the use of water as a reagent is intended.

NOTE – 'Pure chemicals' shall mean chemicals that do not contain impurities, which may affect the result of analysis.

9. Compliance

9.1 When on testing, each of the samples is found to conform to the requirements specified in this Bangladesh Standard Specification, the lot, batch or consignment from which the samples have been drawn shall be deemed to comply with standard specification.

Annex A

(Clause 2.1)

List of Relevant Standards

BDS and ISO No.	Title
BDS 103	Methods of rounding off numerical value
BDS 822	Code of hygienic conditions for food processing units
BDS 833	Water for laboratory use
BDS 1584	Methods of sampling and test for oils and fats, Part-II Purity Tests
BDS ISO 660	Animal and Vegetable fats and oils – Determination of acid value and acidity
BDS ISO 662	Animal and Vegetable fats and oils – Determination of moisture and volatile matter content
BDS ISO 3657	Animal and Vegetable fats and oils – Determination of saponification value
BDS ISO 3596	Animal and Vegetable fats and oils – Determination of unsaponifiable matter – Method using diethyl ether extraction
BDS ISO 3960	Animal and Vegetable fats and oils – Determination of peroxide value – Iodometric (visual) endpoint determination
BDS ISO 3961	Animal and Vegetable fats and oils – Determination of Iodine Value
BDS ISO 5555	Animal and Vegetable fats and oils – Sampling
BDS ISO 6320	Animal and Vegetable fats and oils – Determination of Refractive index
BDS ISO 6883	Animal and Vegetable fats and oils – Determination of conventional mass per volume (litre weight in air)
BDS ISO 10539	Animal and vegetable fats and oils — Determination of alkalinity
BDS ISO 12966-4	Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters – Part 4: Determination by capillary gas chromatography
BDS ISO 15267	Animal and vegetable fats and oils — Flashpoint limit test using Pensky-Martens closed cup flash tester
BDS ISO 15305	Animal and vegetable fats and oils - Determination of Lovibond colour
ISO 17932:2011	Palm oil — Determination of the deterioration of bleachability index (DOBI) and carotene content

BDS ISO 16050	Foodstuffs — Determination of aflatoxin B_1, and the total content of aflatoxins B_1, B_2, G_1 and G_2 in cereals, nuts and derived products — High-performance liquid chromatographic method
BDS ISO 18363-4	Animal and vegetable fats and oils — Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS — Part 4: Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS
BDS ISO 18609	Animal and Vegetable fats and oils – Determination of unsaponifiable matter – method using hexane extraction

Annex B

(*Clause 4.4*)

Gas Liquid Chromatography (GLC) fatty acid composition (expressed as percentages)

Table B.1 — GLC Fatty acid composition for edible palm olien

Carbon Configuration	Composition (expressed as percentage of total fatty acids)
C12:0	0.1 – 0.5
C14:0	0.5 – 1.5
C16:0	38.0 – 43.5
C16:1	ND – 0.6
C17:0	ND – 0.2
C17:1	ND – 0.1
C18:0	3.5 – 5.0
C18:1	39.8 – 46.0
C18:2	10.0 – 13.5
C18:3	ND – 0.6
C20:0	ND – 0.6
C20:1	ND – 0.4
C20:2	ND
C22:0	ND – 0.2
ND – non detectable, defined as 0.05%	

Annex - C

[*Table-1, item (x)*]

Determination of Vitamin – A (as Retinyl palmitate or retinyl acetate)

Method-I

C-1 Principle

C-1.1 The content of vitamin A in fortified edible palm oil is determined by measurement of ultraviolet absorption spectrum of a fraction in which the vitamin A alcohol is collected after its isolation by chromatography. It is expressed in International Unit per gram. One International Unit (I.U) of vitamin A is equivalent to 0.3 μg of vitamin A alcohol or 0.344 μg of vitamin A acetone.

C-2 Reagents

C-2.1 Potassium Hydroxide - 50 percent aqueous solution.

C-2.2 Ethanol- 96 percent (v/v).

C-2.3 Diethyl Ether- Make the diethyl ether free from peroxide by distillation over KOH. Store the peroxide-free ether over coarse granular carbon.

C-2.4 Petroleum Ether-Distilled over KOH, boiling range 40 ºC to 60 ºC.

C-2.5 Activated Alumina - Heat the alumina at 600 ºC for 6 hours cool, sieve through a 180 mm sieve, and add about 3 percent water. Mix thoroughly, and allow the product to stand for at least 12 hours before use. Store in an airtight bottle.

C-2.6 Alkaline Alumina- The alumina (B-2.5) is treated with sodium hydroxide as follows.

C-2.6.1 Stir l0g of alumina (B-2.5) with a solution of l g of sodium hydroxide in 10 ml of water. Allow to stand at room temperature for one hour in a closed bottle and shake occasionally. Heat in a dish in a vacuum drying oven at 100 °C and 20 mm Hg for two and a half hours. Pour the dried product into a bottle without removing any powder clinging to the wall of the dish and stopper securely. To the cool powder add 2 percent water and mix thoroughly. Allow to stand for 18 hours and determine moisture content after drying for an hour in a drying oven at 105 ºC; if lower than 2 percent, again add water, mix etc.

C-2.7 Antimony Trichloride Solution - With a porcelain spoon introduce 65 g of antimony trichloride into a 500 - ml conical flask and wash several times with 15 ml of chloroform (sec B-2.7.1) until the chloroform remains clear. Dissolve the antimony trichloride in 200 ml of chloroform by refluxing. Transfer the warm solution to a bottle containing anhydrous sodium sulphate. After some days antimony trichloride crystals are formed at the bottom and the wall; the solution is then quite clear and ready for use.

C-2.7.1 The chloroform to be used, should be washed and dried with water before use, to remove any alcohol which may have been added to improve keep ability.

C-2.8 Nitrogen-Oxygen-free.

C-3 APPARATUS

C-3.1 Conical Flask- capacity 50 ml.

C-3.2 Separating Funnel- capacity 500 ml.

C-3.3 Round Bottom Flask- capacity 100 ml, with ground glass stopper and two condensers.

C-3.4 Chromatographic Apparatus- The apparatus consists of two parts, each containing a chromatographic column, which may readily by connected in series by means of a rubber stopper connection. The upper tube contains alumina and the lower tube alkaline alumina. Close both the tubes at the bottom with a plug of cotton wool. Fill the longer tube with petroleum ether to a level which reaches into the widened section and then very regularly and gently pour out the alumina at a column of height 15 cm. Fill the shorter column shortly before use in the same way to a height of 2 cm with alkaline alumina.

C-3.5 Pipette - 1 ml capacity, provided with a fine tip.

C-3.6 Tubes - calibrated at 1 ml.

C-3.7 Graduated Flask -10 ml capacity.

C-3.8 Ultraviolet Spectrophotometer.

C-4 Procedure

C-4.1 Saponification of the material.

C-4.1.1 Put l0g of the material into the flask (B-3.3). Add 8 ml of potassium hydroxide solution (B-2.1) and 25 ml of ethanol (B-2.2). Heat gently on the water bath at 58 °C to 90 °C for 15 minutes,

with the reflux condenser attached to the flask (B-3.3). During this procedure a slow current of oxygen- free nitrogen is pass through the liquid.

C-4.2 Extraction of Vitamin A

C-4.2.1 Add 50 ml of distilled water through the condenser and cool the soap solution in the tap water. Transfer the solution into a separating funnel, using another 50 ml of distilled water to rinse the flask. Extract the soap solution with successive portions of 100 ml, 50m1 and 50m1 of diethyl ether by shaking. Wash the combined extract in another separating funnel four times with 50 m1 of distilled water, the first time only by swirling and the following three times by gently shaking. Continue washing if the ether layer is still turbid.

C-4.3 Evaporation of the solvent

C-4.3.1 Use the same flask (B-3.3) in which saponification was carried out. Add the diethyl either solution (B-2.3) in two portions. Heat on the water-bath at 80 ºC to 85 ºC. During distillation, maintain a weak current of oxygen - free nitrogen until approximately 5 ml are left. Transfer the residue to 50 ml of nitrogen and some acetone and again evaporate the solvent.

C-4.3.2 Immediately take the residue in 1 ml to 2 m1 of petroleum ether (if not clear, dry again with acetone) and introduce the solution on the top of the upper chromatographic column with as little petroleum ether (5 ml) as possible.

C-4.4 Chromatographic separation of Vitamin A Alcohol

C-4.4.1 The chromatographic separation is carried out in two stages. In the first stage only the upper column containing alumina (see B-3.4) is used. After the petroleum ether has been brought on to the column, it is passed through the absorbent. Rinse the conical flask with 5 m1 of petroleum ether and bring this quantity on the column. Elute with 5 m1 portions of petroleum ether containing 4 %, 8 % and 12 % diethyl ether (v/v) respectively as previously used for rinsing the conical flask. Discard all these elutes containing substances which are less strongly absorbed than vitamin A alcohol.

C-4.4.2 Test for vitamin A with antimony trichloride in the eluate containing 12 percent diethyl ether if the reaction is positive (in which the alumina contains too much water), repeat the determination with a fresh column packing.

C-4.4.3 Connect the second column containing alkaline alumina (see B-3.4) and elute with 5 m1 each of petroleum ether containing 16 %, 20 % and 24 % diethyl ether (v/v) respectively. Finally use petroleum ether containing 36 percent di-ethyl ether (v/v) until vitamin A eluted completely (see B--4.4.2.1). Collect the eluted fractions in the tubes (B-3.6) mix thoroughly the contents of each tube in order to obtain a homogeneous solution by blowing some air bubbles through the solution by means of a fine-tipped pipette, remove with the same pipette from each of the tubes approximately 0.3 ml carry out Carr-price spot test with these samples. (Use a small test tube 0.5 m1 of antimony trichloride solution and one drop of acetic anhydride). Discard fractions in which the Carr-price spot test is negative generally with 16 % and 20 % diethyl ether (v/v).

C-4.4.3.1 Ensure that during elution the columns do not run dry, but at the same time prevent as much as possible the various petroleum ether fractions from mixing on the top of the first column. Only very little eluate originating from the first column may be present on the top of the second column.

C-4.5 Spectrophotometric determination of Vitamin A

C-4.5.1 Pipette exactly 0.5 ml from each tube in which the Carr-price spot test is positive. Pour these into a 10 m1 graduated flask, make up to the mark with petroleum ether and mix. Make sure that absorption at 326 µm of the petroleum ether used does not change when 10 percent diethy1 ether is added. Use petroleum ether in the blank cell. Read the optical density on the top of the extinction curve (between 324 µm and 326 µm), using a l cm cell (see B-4.5.1.1)

C-4.5.1.1 It is recommended to occasionally compare the shape of the optical density curve between 270 µm and 370 µm with Morton Stubb's ideal curve. This is done to establish whether the separating capacity of the columns is sufficient. Thus the freshly prepared absorbents should always be tested in this way.

C-5 Calculation

$$\textbf{C-5.1}\ \text{Vitamin A, IU/g} = \frac{366a}{w}$$

Where,

w = mass (weight) in g of the material, and
a = maximum optical density.

Method- II

C-0 HPLC Method

C-1 Name of the Material: Vitamin A (as Retinyl palmitate or retinyl acetate) in Oil

C-2 Chromatographic Conditions:

Column	: Luna C_{18}, (150 x 4.6 mm)
Detector	: UV
Mobile Phase	: Methanol: Acetonitrile = 50:50
Wave length	: 325 nm
Flow rate	: 2.0 ml/min.
Column Oven Temperature	: 40 ºC
Injection Volume	: 10 µl
Run Time	: 20 minutes

C-3 Sample and Standard Preparation:

C-3.1 Preparation of Mobile Phase – Take 250 ml methanol and 250ml acetonitrile in a 500 ml volumetric flask. Sonicate it in a ultrasonic bath for 15 minutes and then pass through a 0.22 µm pore size nylon filter.

C-4 Preparation of Standards:

C-4.1 Stock standard solutions: 100 mg of SRM of retinyl palmitate or retinyl acetate was taken in a 100 ml volumetric flask and dissolved in 5 ml Dichloromethane and then made up to the volume with Mobile Phase.

C-4.2 Working standard solutions: Take 1 ml of stock solution in 100 ml volumetric flask and then made up to the volume with mobile phase.

C-5 Sample preparation and Extraction: Take 2 g of oil was taken in 100 ml volumetric flask. The sample was extracted with dichloromethane, MeOH and ACN mixture. An amount 5.0 ml of dichloromethane and 95 ml of mobile phase were added. The sample was shaken in a ultrasonic bath at 40 ºC for 30 minute. Then passed this solution through a C_{18} SPE cartridge to isolate the compound of interest from the sample matrix. Then the eluted solution was evaporated to dryness on a rotary evaporator. The residue was dissolve in 5.0 ml of dichloroethane and taken it in a 100 ml volumetric flask and made up to the volume with mobile phase. The mixture was stirred for 10 minutes and then centrifuged the mixture for 30 minutes. The clear organic top layer was removed and passed through a 0.22 µm pore size syringe filter before injection into HPLC.

C-6 CALCULATION:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Wt. of standard}}{\text{Wt. of Sample}} \times \text{Dilution Factor} \times \text{Potency}$$

Annex D

[*Table 1, Sl. No. (xiv)*]

Determination of Hexane Residues in Oils and Fats

D-1 Principle

The residual hexane content is the quantity of volatile hydrocarbons remaining in the fats and oils following processing involving the use of solvents. 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 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/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.

D-2 Apparatus

D-2.1 Gas Chromatograph - Gas chromatograph having,

a) thermostatic column capable of maintaining the desired column temperature with in ± 1°C;
b) sample inlet system, separately thermostated 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; and
c) flame ionization detector which can be separately thermostated and maintained at a minimum of 100°C.

D-2.2 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 else use electronic integrator (see B-2.3)

D-2.3 Electronic Integrator, which permits rapid and accurate calculations.

D-2.4 Chromatographic Column, either packed or capillary column with the following minimum requirements:

a) *Packed column* - Stainless steel or glass, approximately 2 m long and 3.175 mm internal diameter with acid washed and silanized diatomaceous earth, 150-180 mm particle size (80-100 mesh chromosorb WAW is suitable), stationary phase - Squalene consisting of 10 percent of packing.

b) *Capillary column* - Glass or fused silica approx 30 m long and 0.3 mm internal diameter.

c) *Stationary phase* - Methyl polysiloxane (film thickness 0.2 mm).

D-2.5 Syringe - 1 ml, 10 m1, 1 000 ml capacity, gas tight.

D-2.6 Septum Vial - 20 ml capacity.

D-2.7 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).

D-2.8 Tongs, suitable for holding septum vials.

D-2.9 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.

D-2.10 Shaking Machine

D-3 Reagents

D-3.1 Gases

a) *Carrier* - Helium (preferred for better resolution) or Nitrogen 99.99 percent pure,

b) Flame Ionization Detector - Hydrogen, minimum purity 99.95 percent, air or oxygen, dry, hydrocarbon free (less than 2 ppm hydrocarbon equivalent to CH_4).

D-3.2 Technical Hexane or Light Petroleum, with a composition similar to that used in industrial extraction or failing these n-hexane. For calibration, technical extraction hexane is preferred.

D-3.3 n-Heptane (Internal standard) - analytical reagent grade.

D-3.4 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 percent).

D-4 Sampling and Sample Storage

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.

D-5 GC Operating Conditions

Carrier gas flow depends on the carrier gas and the type of column being used for analysis and should be optimized accordingly. 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 120°C. The column should be maintained at 40°C.

D-6 Procedure

D-6.1 Determination of the Calibration Factor

Weigh to the nearest 0.01 g, 5 g of solvent free vegetable oil (see B-3.4) 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 (in the vial with no added solvent is the blank) according to the following table:

ml/5g	0.5	1	2	4	7	10
mg/100g	67	134	268	536	938	1 340

One vial remains without the addition of solvent.

If *n*-hexane is used for calibration the following table applies:

ml/5g	0.5	1	2	4	7	10
mg/100g	66	132	264	528	924	1 320

Shake the 6 vials containing the solvent in the shaking machine vigorously for 1 h. Using the syringe add 5 ml of internal standard (*see* B-3.3) to each of the 7 vials. Successively immerse the vials up to the neck in the heating bath at 80°C at intervals of approximately 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). The samples must be placed in the heating at intervals such that each sample is tempered for exactly 60 min.

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. Inject immediately into the gas chromatograph.

For each of the vial containing added solvent a calibration factor *F* may be determined by the following 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 are 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.

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.

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 ml of hexane is significantly below the mean value, this deviation is probably due to difficulty in introducing exactly 0.5 ml and this determination must be either eliminated or repeated. For quantities of hexane between 10 and 20 mg/kg it is better to prepare calibration standards by adding 2 ml of internal standard instead of 0.5 ml.

D-6.2 Sample Analysis

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. Using a syringe add through the septum exactly 5ml 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. 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. Immediately inject into the gas chromatograph. Carry out two determinations in rapid succession on each sample.

D-7 Calculation

The residual solvent expressed, in mg/kg (ppm), is given by the following 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 expressed, in mg/kg, of the oil.

F = calibration factor obtained in procedure.

NOTE – For an addition of 5 ml of heptane/5 g of sample
C1 = 680 mg/ kg and C1 = 750 mg/kg, if cyclohexane is used.

Report as the final result the mean of the results of two determinations.