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नेपाल गुणस्तर

NEPAL STANDARD

Soybean Oil - Specification

Government of Nepal

Ministry of Industry, Commerce and Supplies

Nepal Bureau of Standards and Metrology (NBSM)

Kathmandu, Nepal

www.nbsm.gov.np

1. SCOPE

This standard prescribes requirements and methods of sampling and test for soybean oil used for edible purposes and for manufacture of Vanaspati oil and refined oil.

2. REFERENCES

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

NS 212: 2046 Methods of Sampling and Tests for Oils and Fats

NS 212: 2047 Part 1 - Sampling, Physical and Chemical Tests

NS 212: 2046 Part 2 - Purity Tests

ISO 14718:1998 Animal feeding stuffs — Determination of aflatoxin B1 content of mixed feeding stuffs — Method using high-performance liquid chromatography

NS/ISO 2719: 2016 Determination of Flash Point - Pensky-Martens Closed Cup Method

NS*** : Food Colours — Methods of Sampling and Test

3. DEFINITION

For the purpose of this standard, the definitions given in NS 212 (Part 1) and also the following shall apply.

3.1 Refined Soybean Oil — Refined soybean oil means oil which is obtained by solvent extraction of soybean oil bearing materials and processes involving degumming, neutralization, bleaching, deodorization/ deacidification by physical refining.

4. GRADES

4.1 The material obtained by the process of solvent extraction shall be of following grades:

a) Refined,
b) Degummed, and
c) Grade I.

4.1.1 Refined soybean oil is suitable for direct edible consumption.

4.1.2 Degummed and Grade I soybean oil are suitable for making Vanaspati oil and refined oil only and not for direct edible consumption.

5. REQUIREMENTS

5.1 Description

The material shall be obtained from good quality soybeans from the plant *Glycine max* (L) Merrill Syn. *Glycine Soja* Sieb and Zucc., fam. Leguminosae by a process of solvent extraction.

5.1.1 Refined oil and Grade I oil shall be obtained from the oleaginous material using solvent hexane (Food Grade).

5.2 The material shall be clear and free from adulterants, sediment, suspended and other foreign matter, separated water, and added colouring and flavouring substances. The material shall have acceptable taste and odour and when tested as prescribed in **NS 212 (Part 1)**, the peroxide value of the oil shall not exceed 10 milliequivalents peroxide oxygen per kg.

5.2.1 The clarity of the material shall be judged by the absence of turbidity after keeping the filtered sample at 30°C for 24 h.

5.3 Oils shall be free from non-edible oils and adulterants when tested in accordance with **NS 212 (Part 2)**.

5.4 Oil shall not contain aflatoxin, more than 30µg/ kg, when tested by the method prescribed in ISO 14718 or as prescribed in Annex A.

5.5 Metal contaminants and pesticide residues shall not exceed the tolerance limits as prescribed in the *Mandatory Quality Standards of Food* published by Government of Nepal.

5.6 The material shall also comply with the requirements given in Table 1.

5.7 The product shall not contain any of the toxic metals in excess of the quantities prescribed in Table 2.

Table 1. **Requirements for Soyabean Oil**

S.N.	**Characteristic**	Requirement for Grade			Method of Test, Ref to
		Refined	Degummed	Grade I	
1.	Moisture and insoluble impurities, percent by mass, Max	0.10	0.5	0.5	NS 212 (Part 1)
2.	Color on the Lovibond Scale, Expressed as (Y+5R) not deeper than	25[1)]	40[2)]	40[2)]	NS 212 (Part 1)
3.	Refractive Index at 40°C	1.4650 to 1.4710			NS 212 (Part 1)
4.	Saponification value	189 to 195			NS 212 (Part 1)
5.	Iodine value (Wijs)	120 to 140			NS 212 (Part 1)
6.	Acid Value, Max	0.5	2.5	2.5	NS 212 (Part 1)

7.	Unsaponifiable matter, percent by mass, Max	1.0	1.2	1.5	NS 212 (Part 1)
8.	Flash point, Pensky-Martens (closed), °C, Min	250	100	100	NS/ISO 2719: 2016
9.	Hexane, ppm, Max	5.00	-	-	Annex B
10.	Phosphorous Content, ppm, Max	12	200	-	Annex C
11.	Insoluble bromide test	To pass the test			Annex D
[1)] in a 5 ¼ inch cell [2)] in a ¼ inch cell					

Table 2. Limits for Toxic Metals

S. N.	Characteristic	Requirement	Method of Test, Ref to
1.	Lead, mg/kg, *Max*	0.1	NS*** (Food Colours — Methods of Sampling and Test)
2.	Arsenic, mg/kg, *Max*	0.1	
3.	Cadmium, mg/kg, *Max*	1.0	
4.	Mercury (total) mg/kg, *Max*	0.25	

6. PACKING

6.1 The soybean oil shall be packed in any container as prescribed in NS 72 or NS 360 or NS 131 or any suitable well closed containers as agreed to before the purchaser and supplier. Tin or plastic containers once used, shall not be re-used for packaging of edible oils and fats.

6.2 Types and grades which are not suitable for direct edible consumption shall not be packed in consumer packs.

7. MARKING

7.1 The containers shall be marked in English or Nepali language with the following information:

a) Name, type and grade of the material;

b) Net mass in the container;

c) Manufacture's name and address and his recognized trade-mark, if any;

d) Batch no, or lot no, in code or otherwise; and

e) Month and year of manufacture

f) Any other requirement as stipulated under *Food Safety and Quality Act, 2081* and Regulations framed thereunder and *Standard Measurement and Weight Act*, 2025 and rules framed thereunder.

7.2 In addition in the case of the types and grades which are not suitable for direct edible consumption (see **4.1.2**), the following information shall be suitably marked, either printed on the label affixed to the container or lithographed or stencilled thereon with indelible ink, in a type size of not less than 50 mm:

'NOT FOR DIRECT EDIBLE CONSUMPTION'

Types and grades which are not suitable for direct edible consumption shall not be packed in consumer packs.

7.3 The package, label or the advertisement of edible oils and fats shall not use the expressions "Super-Refined", "Extra-Refined", "Micro-Refined", "Double-Refined", "Ultra-Refined", "Anti-Cholesterol", "Cholesterol Fighter", "Soothing to Heart", "Cholesterol Friendly", "Saturated Fat Free" or such other expressions which are an exaggeration of the quality of the product.

7.4 NS Certification Marking

The product may also be marked with the Standard Mark.

The use of the Standard Mark is governed by the provisions of the Nepal Standards (Certification Mark) Act, 2037 and the Rules and Regulations made there under. The details of conditions under which a license for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Nepal Bureau of Standards and Metrology.

8. SAMPLING

8.1 Representative samples of the material shall be drawn as given in **NS 212 (Part 1**).

ANNEX A

DETERMINATION OF TOTAL AFLATOXIN BY ELISA

A-1 PRINCIPLE

Antibodies specific to aflatoxins B1, B2 and G1 are immobilized on the filter, and toxin (aflatoxin B1) is labelled with an enzyme (horseradish peroxidase). Binding of toxin-enzyme conjugate by immobilized antibodies is inhibited by addition of free toxin present in the test sample. Bound enzyme catalyses oxidation of substrate to form a blue complex. Development of colour indicates that the test sample contains aflatoxin.

A-2 APPARATUS

A-2.1 Antibody Coated Solid Support

A-2.2 Aflatoxin Enzyme Conjugate

A-2.3 High Speed Blender

A-2.4 Variable 100-1 000 µl Micropipettes

A-2.5 Glass Culture Tubes

A-2.6 Filters

A-2.7 Timer

A-2.8 Silicon Carbide Boiling Chips

A-3 REAGENTS

A-3.1 Wash Solution — Phosphate Buffered Saline Solution — Dissolve 0.23 g $NaH_2PO_4.H_2O$, 1.95 g $K_2HPO_4.3H_2O$, 8.70 g NaCl, 0.125 ml Tween 20 and 10 mg thimerosal in 900 ml H_2O, adjust *p*H to 7.2 and dilute to 1 l.

A-3.2 Buffer — 0.1 percent Bovine serum albumin in phosphate buffer saline solution containing 0.05 percent thimerosal.

A-3.3 Substrate Solution A, tetramethylbenzidine (TMB), (0.4 g/l H_2O), *p*H 8.3.

A-3.4 Substrate Solution B, hydrogen peroxide (0.02 percent H_2O_2 in 0.13 percent aq. Citric acid solution, *p*H 3.0).

A-3.5 Methanol

A-3.6 Hexane

A-3.7 Chloroform

A-3.8 NaH_2PO_4

A-3.9 K_2HPO_4

A-3.10 NaCl

A-3.11 Tween 20

A-3.12 Bovine Serum Albumin

A-4 PROCEDURE

A-4.1 Preparation of Sample

A-4.1.1 Weigh 50 g of sample into blender jar.

A-4.1.2 Mix with 250 ml of 55 percent methanol and 45 percent water.

A-4.1.3 Mix 100 ml hexane and blend for 1 min at high speed.

A-4.1.4 Filter mixture and recover filtrate.

A-4.1.5 Leave for 5 min and remove the lower phase containing methanol water (A-4.1.2).

A-4.2 Testing

A-4.2.1 Bring all reagents at room temperature (20-23°C).

A-4.2.2 Prepare fresh substrate in small culture tubes by mixing 500 µl substrate solution A with 500 µl substrate solution B for each reaction sites used.

A-4.2.3 Add 100 µl test extract to 200 µl buffer (A-3.2).

A-4.2.4 Thoroughly mix the diluted test extract and apply 100 µl diluted test extract to the centre of membrane. Using timer, wait for 1 min.

A-4.2.5 Apply 100 µl (2 drops) enzyme solution to the centre if membrane. Using timer, wait for 1 min.

A-4.2.6 Wash with 1.5 ml (30 drops) wash solution added drop wise.

A-4.2.7 Add the entire content of the substrate solution

1.0 ml from each test tube to each reaction site. Wait 1 min and immediately observe site (centre of cup) for blue colour development (negative) or no colour development (positive).

A-4.3 Interpretation of Results

A-4.3.1 Observe the reaction site (centre of the cup) for a blue colour or no colour development at exactly 1 min after adding the substrate A and B mixture (A-3.3 and A-3.4).

Negative — If the reaction site (centre of the cup) turns light blue or darker, test sample contains total aflatoxin B1, B2 and G1.

Positive — If no blue colour is observed in the reaction site (centre of cup) and reaction site remains completely white (no colour change) for at least 1 min, the test sample contains aflatoxin B1, B2 and G1.

ANNEX B

DETERMINATION OF HEXANE RESIDUES IN OILS AND FATS

B-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. It is suitable for determination of quantities of hexane between 10 and 1 500 mg/kg in fats and oils.

B-2 APPARATUS

B-2.1 Gas Chromatograph

Gas chromatograph having thermostatic column capable of maintaining the desired column temperature with in ± 1°C; 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 flame ionization detector which can be separately thermostated and maintained at a minimum of 100°C.

B-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 (B-2.3)

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

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

Packed Column — stainless steel or glass, approx 2 m long and 3.175 mm internal diameter with acid washed and silanized diatomaceous earth, 150-180 µm particle size (80-100 mesh Chromosorb WAW is suitable), stationery phase — squalene consisting of 10 percent of packing.

Capillary Column — glass or fused silica approx 30 m long and 0.3 mm internal diameter.

Stationery phase — Methyl polysiloxane (film thickness 0.2 µm).

B-2.5 Syringe — 1 µl, 10 µ1, 1000 µl capacity, gas tight.

B-2.6 Septum Vial — 20 ml capacity.

B-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.)

B-2.8 Tongs, suitable for holding septum vials

B-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.

B-2.10 Shaking Machine

B-3 REAGENTS

B-3.1 Gases

Carrier — Helium (preferred for better resolution) or Nitrogen 99.99 percent pure, dried and containing a maximum of 10 mg O_2/kg.

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

B-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.

B-3.3 *n*-Heptane — (internal standard) analytical reagent grade.

B-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).

B-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.

B-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.

B-6 PROCEDURE

B-6.1 Determination of the Calibration Factor

Weigh to the nearest 0.01 g, 5 g of solvent free vegetable oil (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:

µl/5g	0.5	1	2	4	7	10
µg/100g	67	134	268	536	938	1340

One vial remains without the addition of solvent.

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

µl/5g	0.5	1	2	4	7	10
µg/100g	66	132	264	528	924	1320

Shake the 6 vials containing the solvent in the shaking machine vigorously for 1 h. Using the syringe add 5 µl of internal standard (B-3.3) to each of the 7 vials. Successively immerse the vials upto the neck in the heating bath 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). 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 1 000 µ1(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 cyclo-pentane, 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.

Cs = 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 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. 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.

B-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 5µl 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 1 000 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.

B-7 CALCULATION

The residual solvent expressed in mg/kg (ppm) is given by the following formula:

$$W = \frac{(A_H - A_1) * F * C_1}{A_1}$$

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 C_1 = 680 mg/kg and C_1 = 750 mg/kg if cyclohexane is used.

F = calibration factor obtained in procedure

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

ANNEX C

DETERMINATION OF PHOSPHORUS

C-1 PRINCIPLE

The test portion is ignited and the ashes so obtained are treated with nitric acid. Ammonium molybdate reacts with phosphorous under acidic conditions to form molybdophosphoric acid, which in presence of vanadium forms yellow phosphovanadomolybdic complex. The intensity of yellow colour is proportional to phosphate concentration.

C-2 APPARATUS

C-2.1 Calibrated Pipettes, 5 ml and 20 ml.

C-2.2 Crucible or Porcelain Evaporating Dish, 40- 50 ml.

C-2.3 Colorimeter or Spectrophotometer, suitable for observations at 460 nm.

C-2.4 Furnace, giving a temperature of 800 to 900°C.

C-3 REAGENTS

C-3.1 Magnesium Oxide, light, pure, free from phosphorus.

C-3.2 Nitric Acid, approximately 6N aqueous solutions.

C-3.3 Ammonium Molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$], analytical reagent quality, 50 g/1 aqueous solution.

C-3.4 Acid Aqueous Solution of Ammonium Vanadate [NH_4VO_3] Dissolve about 2.5 g of ammonium vanadate in about 500 ml of hot water. Cool, and then add about 20 ml of nitric acid (ρ = 1.33g/ml). Make up to 1 litre with distilled water.

C-3.5 Standardized Solution Containing 1 mg of Phosphorus per ml Dissolve 1.156 (± 0.001) g of disodium hydrogen phosphate [Na2HPO4, 12 H2O], or 0.439 3 (± 0.000 5) g of mono-potassium hydrogen phosphate [KH2PO4] in exactly 100 ml of distilled water.

C-4 PROCEDURE

C-4.1 Calibration Dilute the standardized solution (C-3.5) in such a mane as to obtain solutions containing exactly 0.4, 0.2, 0.1, 0.05, and 0.01 mg of phosphorus per ml. Utilize these solutions in order to plot the calibration curve of the instrument (C-2.3) in such a way that this curve expresses the results in mg of phosphorus per ml of the final solution.

C-4.2 Determination Weigh to within 1 mg exactly 0.1 g of magnesium oxide(C-3.1) into the crucible or porcelain dish (C-2.2), and ignite. Allow to cool, then weigh, to within 1 mg about 0.1 to 10 g of fat, according to its presumed phosphorus content. Burn of the fat (if necessary with the assistance of a folded, ash less filter paper). Ignite to a white ash in the furnace (C-2.4) at 800 to 900°C. Dissolve the magnesium containing ash in exactly 5 ml of the aqueous nitric acid solution (C 3.2) with the aid of a 5 µl pipette (C-2.1). Add exactly 20 ml of a mixture of 10 ml of the

aqueous ammonium molybdate solution (C-3.3) and 10 ml of the acid aqueous ammonium vanadate solution (C-3.4). Mix and allow to stand for 20 min. Prepare a blank test, not containing fat, under exactly the same conditions. Transfer the test solution into the cell of the apparatus (C-2.3), Measure the extinction at 460 nm against the blank solution. Read the absorbance (or other indications on the scale of the colorimeter).

C-5 EXPRESSION OF RESULTS

With the aid of the calibration curve obtained according to C-4.1 and absorbance or other figure read on the instrument, read from the graph the amount of phosphorus (m1) in mg per ml of test solution.

The percentage (m/m) of phosphorus present in the oil is given by the following formula:

$$\text{Phosphorous content (percent)} = \frac{2.5\, m_1}{\text{m}}$$

Where,

m_1 = concentration of phosphorus read from the curve, in mg per ml; and

m = mass of the test portion, in g.

ANNEX D

TEST FOR INSOLUBLE BROMIDES

D-1 PROCEDURE

Dissolve in a test tube about 6 g of the material in 12 ml of a mixture of equal parts of chloroform and acetic acid. Add bromine, dropwise, until a slight excess is indicated by the colour, keeping the solution at 20°C. Allow the mixture to stand for at least 15 min and place the test tube in boiling water.

D-2 RESULT

The material shall be taken to have passed the test if the solution when kept in boiling water, does not become cloudy on account of insoluble bromides.