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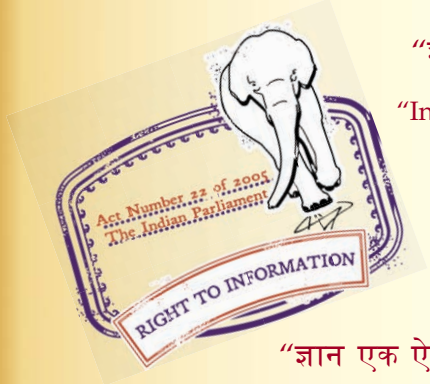
IS 4941 (1994): Extracted Honey [FAD 3: Apiary Industry]



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"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

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IS 4941 : 1994
(Reaffirmed 2002)

भारतीय मानक

शहद – विशिष्टि

(दूसरा पुनरीक्षण)

Indian Standard

EXTRACTED HONEY — SPECIFICATION

(Second Revision)

UDC 638·16

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

May 1994

Price Group 5

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Apiary Industry Sectional Committee had been approved by the Food and Agriculture Division Council.

This standard was published in 1968 and was first revised in 1974. The present revision incorporates the following modifications:

a) the definition of honey has been modified; and

b) the aniline chloride test has been withdrawn and the photometric determination of hydroxymethyl furfural (HMF) content has been included.

c) Honey not complying with requirements as given is Sl No. (ix) of Table 1 and having any objectionable flavour, aroma or taint and fermented due to processing, storage or handling must be labelled as 'Industrial Honey'.

While formulating this specification, the Committee took into consideration the prevailing trade practices and the different grades prescribed by the Agricultural Marketing Advisor to the Government of India for incorporation in the General (grading and marking) rules framed under the *Agricultural Produce (Grading and Marking) Act*, 1937.

This standard is also subject to the restrictions imposed under the *Prevention of Food Adulteration Act*, 1954 and Rules framed thereunder, wherever applicable.

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 IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

AMENDMENT NO. 1 APRIL 2011
TO
IS 4941 : 1994 EXTRACTED HONEY — SPECIFICATION

(Second Revision)

[*Page* 1, *clause* 9.1(e)] — Substitute 'Net quantity' *for* 'Net weight'.

[*Page* 1, *clause* 9.1(e)] — Insert the following at the end:

'f) Any other requirement as given under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977'and the *Prevention of Food Adulteration Act*, 1955 and the Rules framed thereunder'.

(FAD 3)

Reprography Unit, BIS, New Delhi, India

Indian Standard

EXTRACTED HONEY — SPECIFICATION

(Second Revision)

1 SCOPE

This standard prescribes the requirements and the methods of sampling and test for extracted honey obtained from honey bees.

2 REFERENCES

The following Indian Standards are necessary adjuncts to this standard :

IS No.	*Title*
460 (Part 1) : 1985	Test sieves : Part 1 Wire cloth test sieves (*third revision*)
460 (Part 2) : 1985	Test sieves : Part 2 Perforated plate test sieves (*third revision*)
1070 : 1992	Reagent grade water (*third revision*)

3 DEFINITION

Honey is the natural sweet substance produced by honey bees from the nectar of blossoms or from secretions of plants which honey bees collect, transform and store in honey combs for ripening.

4 GRADES

4.1 Honey shall be of three grades, namely, Special, Grade A and Standard.

5 REQUIREMENTS

5.1 General Characteristics

It shall be well-ripened natural product. It shall be clear. It shall have been extracted with the help of an extractor. It shall be free from objectionable flavour due to overheating, fermentation and smoke. It shall have been strained clear through a double layered cheese cloth (150 microns) at a temperature not exceeding 70°C.

5.2 Freedom from Foreign Matter

When visually inspected, the honey shall be free from any foreign matter, such as mould, dirt, scum, pieces of beeswax, the fragments of bees and other insects and from any other extraneous matter.

5.3 Colour

The colour of honey shall be uniform throughout and may vary from light to dark brown.

5.4 The honey shall not contain any food additives, such as colour, vitamins, minerals and saccharin.

5.5 The honey of different grades shall also comply with the requirements given in Table 1.

6 SAMPLING

6.1 Representative samples of the honey shall be drawn according to the method prescribed in Annex J.

7 TESTS

7.1 Test shall be carried out as prescribed in the appropriate Annexes specified in col 6 of Table 1.

7.2 Quality of Reagents

Unless otherwise specified, pure chemicals shall be employed in tests and reagent grade water (*see* IS 1070 : 1992) 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 affect the results of analysis.

8 PACKING

8.1 The honey shall be packed in hygienically clean, wide-mouthed, glass containers or in acid-resistant lacquered tin containers or in suitable polyethylene containers. The screwed caps for the glass containers shall be of non-corrosive and non-reactive material to honey and shall be provided with cork washers to avoid spilling.

9 MARKING

9.1 Each container shall be legibly and indelibly marked with the following:

a) Name of the material and grade designation;
b) Name of the packer;
c) Batch or code number;
d) Date of packing; and
e) Net weight.

Table 1 Requirements for Extracted Honey

(*Clause* 5.5)

Sl No.	Characteristic	Special Grade	A Grade	Standard Grade	Method of Test, Ref to Annex
(1)	(2)	(3)	(4)	(5)	(6)
i)	Specific gravity at 27°C, *Min*	1.37	1.37	1.37	A
ii)	Moisture, percent by mass, *Max*	20	22	25	B
iii)	Total reducing sugar, percent by mass, *Min*	70	65	65	C
iv)	Sucrose, percent by mass, *Max*	5.0	5.0	5.0	C
v)	Fructose-glucose ratio[1], *Min*	1.00	1.00	1.00	C
vi)	Ash, percent by mass, *Max*	0.5	0.5	0.5	D
vii)	Acidity (expressed as formic acid), percent by mass, *Max*	0.2	0.2	0.2	E
viii)	Fiehe's test	← Negative →			F
ix)	Hydroxymethyl furfural (HMF), mg/kg, *Max*	80	80	80	F
x)	Total count of pollens and plant elements/g of honey, *Max*	50 000	50 000	50 000	G
xi)	Optical density, at 660 nm, percent, *Max*	0.3	0.3	0.3	H

[1] If Fiehe's test is positive, carry out the determination of hydroxymethyl furfural (HMF) content. If it is more than 80 mg per kg, then fructose glucose ratio should be more than 1.00.

ANNEX A

[*Table* 1, *Item* (i)]

DETERMINATION OF SPECIFIC GRAVITY

A-1 APPARATUS

A-1.1 Thermostatically Controlled Water-Bath — Maintained at 27 ± 1°C.

A-1.2 Specific Gravity Bottle

A-2 PROCEDURE

A-2.1 Clean and thoroughly dry the specific gravity bottle and weigh. Fill it up to the mark with freshly boiled and cooled distilled water which has been maintained at 27 ± 1°C and weigh. Remove the water, dry the bottle again and fill it with the honey sample maintained at the same temperature. Weigh the bottle again.

A-3 CALCULATION

A-3.1 Specific gravity at 27°C = $\frac{C - A}{B - A}$

where

C = mass, in g, of the specific gravity bottle with the honey sample;

A = mass, in g, of the empty specific gravity bottle; and

B = mass, in g, of the specific gravity bottle with water.

ANNEX B

[*Table* 1, *Item* (ii)]

DETERMINATION OF MOISTURE

B-0 Two methods have been specified a) Oven drying method, and b) Refractometer method. Oven drying method should be used as reference method; however, refractometer method may be used for routine analysis of honey with moisture content up to 25 percent.

B-1 OVEN DRYING METHOD

B-1.1 Apparatus

B-1.1.1 *Flat-Bottom Dish* —of nickel or other suitable material not affected by boiling water; 7 cm to 8 cm in diameter and not more than 2.5 cm deep.

B-1.1.2 *Sand*

Passing through 500-micron IS Sieve [*see* IS 460 (Part 1) and (Part 2) : 1985] but retained on 180-micron IS Sieve. It shall be prepared by digestion with concentrated hydrochloric acid, followed by thorough washing with water till free from chlorides. It shall be dried and ignited to dull red heat.

B-1.1.3 *Vacuum Oven*

B-1.2 Procedure

B-1.2.1 Heat the dish containing 20 g of the prepared sand and a stirring rod in the oven for one hour. Allow to cool in an efficient desiccator for 30 to 40 minutes. Weigh accurately 2 g of the material into the tared dish. Add 5 ml of distilled water in the dish and thoroughly mix sand with the sample by stirring with the glass rod having a widened flat end, smoothing out lumps and spreading the mixture over the bottom of the dish.

B-1.2.1.1 Place the dish on a boiling water-bath for 30 minutes. Wipe the bottom of the dish and transfer it, with the glass rod, to the vacuum oven maintained at a temperature between 60°C and 70°C and at a pressure not more than 50 mm of mercury.

B-1.2.1.2 After 2 hours, remove the dish and transfer to a desiccator, allow it to cool and then weigh. Replace the dish in the oven for a further period of one hour, remove and transfer to the desiccator, cool and weigh again. Repeat the process of heating, cooling and weighing after every one hour till consecutive weighings do not differ by more than 0.5 mg.

B-1.3 Calculation

B-1.3.1 moisture, percent by mass $= \dfrac{100\ (M_1 - M_2)}{M_1 - M}$

where

M_1 = mass, in g, of the contents of the dish before drying;

M_2 = mass, in g, of the contents of dish after drying; and

M = mass, in g, of the empty dish with the sand and the glass rod.

B-2 REFRACTOMETER METHOD

B-2.1 Apparatus

B-2.1.1 *Refractometer*

B-2.2 Procedure

Determine the refractometer reading of honey at 20°C and calculate the percentage of moisture from the values given in Table 2. If the determination is made at a temperature other than 20°C, correct the reading according to the Note in Table 2.

Table 2 Relationship Between Refractive Index and Moisture Content of Honey

Refractive Index at 20°C	Moisture, Percent by Mass	Refractive Index at 20°C	Moisture, Percent by Mass
(1)	(2)	(1)	(2)
1.504 4	13.0	1.488 5	19.2
1.503 8	13.2	1.488 0	19.4
1.503 3	13.4	1.487 5	19.6
1.502 8	13.6	1.487 0	19.8
1.502 3	13.8	1.486 5	20.0
1.501 8	14.0	1.486 0	20.2
1.501 2	14.2	1.485 5	20.4
1.500 7	14.4	1.485 0	20.6
1.500 2	14.6	1.484 5	20.8
1.499 7	14.8	1.484 0	21.0
1.499 2	15.0	1.483 5	21.2
1.498 7	15.2	1.483 0	21.4
1.498 2	15.4	1.482 5	21.6
1.497 6	15.6	1.482 0	21.8
1.497 1	15.8	1.481 5	22.0
1.496 6	16.0	1.481 0	22.2
1.496 1	16.2	1.480 5	22.4
1.495 6	16.4	1.480 0	22.6
1.495 1	16.6	1.479 5	22.8
1.494 6	16.8	1.479 0	23.0
1.494 0	17.0	1.478 5	23.2
1.493 5	17.2	1.478 0	23.4
1.493 0	17.4	1.477 5	23.6
1.492 5	17.6	1.477 0	23.8
1.492 0	17.8	1.476 5	24.0
1.491 5	18.0	1.476 0	24.2
1.491 0	18.2	1.475 5	24.4
1.490 5	18.4	1.475 0	24.6
1.490 0	18.6	1.474 5	24.8
1.489 5	18.8	1.474 0	25.0
1.489 0	19.0		

NOTE — Temperature correction for refractive index = 0.000 23 per deg C. If the reading is made at a temperature above 20°C, add the correction; if made below, subtract the correction.

ANNEX C

[*Table* 1, *Items* (iii), (iv) *and* (v)]

DETERMINATION OF TOTAL REDUCING SUGARS, SUCROSE AND FRUCTOSE-GLUCOSE RATIO

C-1 TOTAL REDUCING SUGARS

C-1.1 Reagents

C-1.1.1 *Soxhlet Modification of Fehling's Solution*

Prepare by mixing equal volumes of Solution *A* (**C-1.1.2**) and Solution *B* (**C-1.1.3**) immediately before using.

C-1.1.2 *Copper Sulphate Solution (Solution A)*

Dissolve 34.639 g of copper sulphate crystals ($CuSO_4.5H_2O$) in water, dilute to 500 ml and filter through glass wool or filter paper.

C-1.1.2.1 *Standardization of copper sulphate solution*

Using separate pipettes, pipette out accurately 5 ml of Solution *A* (**C-1.1.2**) and 5 ml of Solution *B* (**C-1.1.3**) into a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard invert sugar solution (**C-1.1.5**) from a burette, about one millilitre less than the expected volume which will reduce the Fehling's solution completely (about 48 ml). Add one millilitre of methylene blue indicator while keeping the solution boiling. Complete the titration within three minutes, the end point being indicated by change of colour from blue to red. From the volume of invert sugar solution used, calculate the strength(s) of the copper sulphate solution by multiplying the titre value by 0.001 (mg/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml of copper sulphate solution.

C-1.1.3 *Potassium Sodium Tartrate (Rochelle Salt) Solution (Solution B)*

Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, dilute to 500 ml. Let the solution stand for a day and filter.

C-1.1.4 *Hydrochloric Acid*

Sp gr 1.18 at 20°C (approximately 12 N).

C-1.1.5 *Standard Invert Sugar Solution*

Weigh accurately 0.95 g sucrose and dissolve it in 500 ml of water. Add 2 ml of concentrated hydrochloric acid, boil gently for 30 minutes and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1 000 ml; 50 ml of this solution contains 0.05 g invert sugar.

C-1.1.6 *Methylene Blue Indicator* — 0.2 percent in water.

C-1.2 Procedure

Place accurately weighed, one gram (*W*) of the prepared sample of honey into a 250-ml volumetric flask and dilute with 150 ml of water. Mix thoroughly the contents of the flask and make the volume to 250 ml with water. Using separate pipettes, take accurately 5 ml each of Solution *A* (**C-1.1.2**) and Solution *B* (**C-1.1.3**), in a porcelain dish. Add 12 ml of honey solution from a burette and heat to boiling over an asbestos gauze. Add one millilitre of methylene blue indicator and while keeping the solution boiling complete the titration, within three minutes, the end point being indicated by change of colour from blue to red. Note the volume (*H*) in ml of honey solution required for the titration.

C-1.3 Calculation

$$\text{Total reducing sugars, percent by mass} = \frac{250 \times 100 \times S}{H \times M}$$

where

S = strength of copper sulphate solution;

H = volume, in ml, of honey solution required for titration; and

M = mass, in g, of honey.

C-2 SUCROSE

C-2.1 Procedure

To 100 ml of the stock honey solution (*see* **C-1.2**), add one millilitre of concentrated hydrochloric acid and heat the solution to near boiling. Keep aside overnight. Neutralize this inverted honey solution with sodium carbonate and determine the total reducing sugars as described in **C-1.2**.

C-2.2 Calculation

C-2.2.1 Sucrose, percent by mass = [(reducing sugars after inversion, percent by mass) – (reducing sugars before inversion, percent by mass)] × 0.95.

C-3 FRUCTOSE-GLUCOSE RATIO

C-3.1 Reagents

C-3.1.1 *Iodine Solution* — 0.05 N.

C-3.1.2 *Sodium Hydroxide Solution* — 0.1 N.

C-3.1.3 *Sulphuric Acid* — concentrated.

C-3.1.4 *Standard Sodium Thiosulphate Solution* — 0.05 N.

C-3.2 Procedure

Pipette 50 ml of honey solution (*see* C-1.2) in a 250-ml stoppered flask. Add 40 ml of iodine solution and 25 ml of sodium hydroxide solution. Stopper the flask and keep in dark for 20 minutes. Acidify with 5 ml of sulphuric acid and titrate quickly the excess of iodine against standard sodium thiosulphate solution. Conduct a blank using 50 ml of water instead of honey solution.

C-3.3 Calculation

C-3.3.1 Approximate glucose, percent by mass $(w) = \frac{(B - S) \times 0.004\,502 \times 100}{a}$

where

B = volume of sodium thiosulphate solution required for the blank,

S = volume of sodium thiosulphate solution required for the sample, and

a = mass of honey taken for test.

C-3.3.2 Approximate fructose, percent by mass $(x) = \frac{\text{Approximate total reducing sugars, percent} - w}{0.925}$

True glucose, percent by mass $(y) = w - 0.012\,x$

True fructose, percent by mass $(z) = \frac{\text{Approximate reducing sugars, percent} - y}{0.925}$

True reducing sugars, percent by mass $= y + z$

C-3.3.3 Fructose-glucose ratio $= \frac{\text{True fructose, percent by mass } (z)}{\text{True glucose, percent by mass } (y)}$

ANNEX D

[*Table* 1, *Item* (vi)]

DETERMINATION OF ASH

D-1 PROCEDURE

D-1.1 Weigh accurately 5 g to 10 g of the honey sample in a silica or platinum dish, add a few drops of pure olive oil to prevent spattering, heat carefully over a low flame until swelling ceases. Ignite in a muffle furnace at 600 ± 20°C till white ash is obtained. Cool the dish in a desiccator and weigh. Incinerate to constant weight.

D-2 CALCULATION

D-2.1 Ash, percent by mass $= \frac{100\,(M_2 - M)}{M_1 - M}$

where

M_2 = mass, in g, of the dish with the ash;

M = mass, in g, of the empty dish; and

M_1 = mass, in g, of the dish with the material taken for the test.

ANNEX E

[*Table* 1, *Item* (vii)]

DETERMINATION OF ACIDITY

E-1 REAGENTS

E-1.1 Standard Sodium Hydroxide Solution — 0.05 N.

E-1.2 Phenolphthalein Indicator Solution — Dissolve 0.5 g of phenolphthalein in 100 ml of 50 percent ethyl alcohol (*v/v*).

E-2 PROCEDURE

E-2.1 Take 10 g of the sample in a suitable titration flask and dissolve it in 75 ml of carbon dioxide-free water. Mix thoroughly. Titrate against standard sodium hydroxide solution using 4 to 6 drops of carefully neutralized phenolphthalein solution (pink colour of indicator should persist for at least 10 seconds). Determine blank on water and indicator and correct the volume of standard sodium hydroxide solution used.

E-3 CALCULATION

E-3.1 Acidity (as formic acid), percent by mass $= \frac{0.23 \times V}{M}$

where

V = corrected volume of 0.05 N sodium hydroxide solution required for titration; and

M = mass, in g, of the sample taken for the test.

ANNEX F

[*Table* 1, *Items* (viii) *and* (ix)]

FIEHE'S TESTS AND DETERMINATION OF HYDROXYMETHYL FURFURAL (HMF) CONTENT

F-0 Carry out the hydroxymethyl furfural (HMF) test (**F-2**) only when Fiehe's test (**F-1**) is positive.

F-1 FIEHE'S TEST

F-1.1 Reagents

F-1.1.1 *Resorcinol Solution*

Dissolve 1 g resublimed resorcinol in 100 ml hydrochloric acid (sp gr 1.18 to 1.19).

F-1.1.2 *Ether* — sulphuric ether.

F-1.2 Procedure

Transfer 5 g of the honey sample into a mortar, using a pestle, mix the honey with 10 ml of ether. Decant the ether extract into a porcelain dish. Repeat the extraction twice in the same manner and collect the extract in the same dish. Allow the extracts to evaporate to dryness at room temperature and add a large drop of freshly prepared resorcinol solution. The production of cherry red colour appearing instantly indicates a positive reaction. Faint pink colour disappearing after a short time or yellow to salmon pink colours indicates a negative reaction.

F-2 DETERMINATION OF HMF CONTENT

F-2.1 Reagents

F-2.1.1 *Barbituric Acid Solution*

Weigh out 500 mg barbituric acid and transfer to a 100 ml graduated flask using 70 ml water. Place in a hot water bath until it dissolves, cool and make up to volume.

F-2.1.2 *p-Toluidine Solution*

Weigh out 10.0 g *p*-toluidine, analytical grade and dissolve in about 50 ml isopropanol by gentle warming on a water bath. Transfer to a 100 ml graduated flask with isopropanol and add 10 ml glacial acetic acid. Cool and make up to volume with isopropanol. Keep the solution in the dark. Do not use the solution for at least 24 hours.

F-2.2 Distilled Water (Oxygen Free)

F-2.2.1 Nitrogen gas is passed through boiling distilled water. The water is then cooled.

F-2.3 Apparatus

F-2.3.1 *Spectrophotometer to Read at 550 nm*

F-2.3.2 *Water Bath*

F-2.4 Sampling

F-2.4.1 The honey is prepared as in **6.1** without any heating.

F-2.5 Procedure

F-2.5.1 *Preparation of Test Sample*

10 g of honey sample is weighed and dissolved without heating in 20 ml oxygen free distilled water. This is transferred to a 50 ml graduated flask and made up to volume (honey solution). The sample should be tested after preparation without delay.

F-2.5.2 *Photometric Determination*

2.0 ml of honey solution is pipetted into each of two test tubes and 5.0 ml *p*-toluidine solution is added to each. Into one test tube 1 ml water is pipetted and into the other 1 ml barbituric acid solution and both mixtures are shaken. The one with added water serves as the water blank. The addition of the reagents should be done without pause and should be finished in about 1 to 2 minutes.

The extinction of the sample is read against the blank at 550 nm using a 1 cm cell, immediately after the maximum value is reached.

F-2.6 Calculation

F-2.6.1 The method may be calibrated by using a standard solution of hydroxymethyl furfural dehyde (HMF) standardized by dissolving commercial or laboratory prepared HMF and assaying spectrophotometrically where $\varepsilon = 16.830$ (J. H. Turner 1954) at 284 nm using 0.300 0 µg standards. An equation by which result may be worked out is given below :

$$\text{mg/100 g HMF} = \frac{\text{Absorbance}}{\text{Thickness of Layer}} \times 19.2$$

Results are expressed as mg HMF/kg honey.

ANNEX G

[*Table* 1, *Item* (x)]

DETERMINATION OF TOTAL COUNT OF POLLENS AND PLANT ELEMENTS IN HONEY

G-1 PROCEDURE

G-1.1 Weigh accurately 10 g of honey in a small clean beaker. Dissolve the honey in 50 ml of distilled water. For honey rich in sediments, the quantity of honey may be reduced to 5 g or 1 g and dilution and calculation may suitably be altered. Transfer this carefully to a 100-ml measuring cylinder and fill the cylinder with distilled water up to 100-ml mark. Centrifuge 10 ml of this stock solution in 15-ml centrifuge tube at 3 000 rev/min for 5 minutes. Decant cautiously the supernatant liquid without disturbing the sediment, taking care to leave one millilitre of the liquid with the sediment in the tube. Then, shake well the sediment and completely transfer to a collecting tube. Repeat centrifuging for all the stock solutions of honey and sediments in the same collection tube. To these sediments in the collection tube add a drop of 0.5 percent alcoholic basic fuchsin solution and stir the sediment well. Then centrifuge it and draw of the supernatant liquid and disperse the sediment in one millilitre of the solution. Shake well the sediment and place a drop of this solution on the one millimetre squares on the haemocytometer and place a coverslip. Count pollens fungal spores and algae present in one millimetre square at a magnification of 100 ×. Repeat this counting ten times and take 10 different counts with the dispersed sediment.

G-2 CALCULATION

G-2.1 The average number of plant elements counted over the haemocytometer are for the volume 0.1 mm (1 mm square × 0.1 mm depth).

From this, calculate the pollens and plant elements present in one millilitre, which is equivalent to their absolute number present in 10 g of honey. Express the result as the number of pollens and plant elements in 1 g of honey.

ANNEX H

[*Table* 1, *Item* (xi)]

DETERMINATION OF OPTICAL DENSITY OF HONEY

H-1 PROCEDURE

H-1.1 Weigh accurately 2 g of honey in a small beaker and dissolve it in distilled water. Make the solution to 10 ml in a 10-ml measuring cylinder. Adjust the colorimeter with distilled water in a cuvet at '0' absorbance or 100 percent transmittance at 660 nm. Take the honey solution in the cuvet and read the absorbance directly or as the percent transmittance at the same wave length. Calculate the optical density by using the following formula, if the colorimeter has been provided with transmittance scale only:

Optical density = 2 – Log percent transmittance.

ANNEX J

(*Clause* 6.1)

SAMPLING OF HONEY

J-1 GENERAL REQUIREMENTS

J-1.0 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

J-1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

J-1.2 The sampling instrument shall be clean and dry when used.

J-1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers for samples from adventitious contamination.

J-1.4 The samples shall be placed in clean and dry glass containers. The sample containers shall be of such a size that they are almost completely filled by the sample.

J-1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, code number and other important particulars of the consignment.

J-1.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

J-2 SCALE OF SAMPLING

J-2.1 Lot

All the containers in a single consignment belonging to the same grade of material shall constitute a lot. If the consignment is declared to consist of different grades of material, the containers belonging to the same grade shall be grouped together and the groups of containers of the same grade in a consignment shall constitute separate lots.

J-2.1.1 Samples shall be tested from each lot for ascertaining its conformity to the requirements of this specification.

J-2.2 The number of containers to be selected from each lot shall depend on the size of the lot and shall be done in accordance with col 1, 2 and 3 of Table 3.

Table 3 Number of Containers to be Selected for Sampling

(*Clause* J-2.2)

Lot Size (*N*)	No. of Containers to be Selected (*n*) for Size of the Container	
	500 g and Above	Below 500 g
(1)	(2)	(3)
Up to 25	3	6
26 to 150	4	6
151 to 500	5	9
501 and above	7	12

J-2.3 The containers shall be chosen at random from the lot and for this purpose a random number table as agreed to between the purchaser and the supplier shall be used. If such a table is not available, the following procedure shall be adopted:

Starting from any container in the lot, count them as 1, 2, 3..., up to r in a systematic manner, where r is equal to the integral part of N/n, N being the total number of containers in the lot, n the number of containers to be chosen (*see* Table 3). Every rth container thus counted shall be separated until the requisite number of containers is obtained from the lot to give samples for test.

J-3 TEST SAMPLES AND REFEREE SAMPLES

J-3.0 The samples shall be drawn and prepared according to **J-3.1** and **J-3.2**, when the containers are selected according to col 2 of Table 3. Clause **J-3.3** shall be followed when the containers are selected according to col 3 of Table 3.

J-3.1 Preparation of Individual Samples

Draw with suitable sampling instrument equal quantities of the material from different parts (top, middle, bottom, etc) of the container till about 300 g of the material is drawn; divide it into three equal parts. Each part so obtained shall constitute an individual sample representing the container and shall be transferred immediately to thoroughly cleaned, dry containers, sealed air-tight, and marked with particulars given under **J-1.5**. Three individual samples so obtained from each container shall be made into sets in such a way that each set has a sample representing each selected container. One of these shall be marked for the purchaser, another for the vendor and the third for the referee.

J-3.2 Preparation of Composite Sample

From the material from each of the selected container, remaining after the individual sample has been taken, approximately equal quantities of material shall be taken and mixed together so as to form a composite sample weighing about 150 g. This composite sample shall be divided into three equal parts and transferred to clean and dry containers, sealed air-tight and labelled with particulars as given in **J-1.5**. One of these composite samples shall be for the purchaser, another for the vendor and the third for the referee.

J-3.3 When honey is in containers of size less than 500 g, the number of containers shall be selected according to col 3 of Table 3. The selected containers shall be divided at random into three equal sets. The containers belonging to each set shall be opened and approxi-mately equal quantity of material shall be taken and mixed together to form a composite sample of 50 g. The honey left in each container after the preparation of composite sample shall be sealed air-tight with all the particulars as given in **J-1.5**. The individual samples also shall be packed in air-tight containers with all the particulars as given in **J-1.5**. The three sets of the individual sample with their corresponding composite sample shall be marked in such a way that one set is for the purchaser, another for the vendor and the third for the referee.

J-3.4 Referee Sample

Referee sample shall consist of a set of individual samples (**J-3.1** and **J-3.3**) and a composite sample (**J-3.2** and **J-3.3**) marked for this purpose and shall bear the seals of the purchaser and the vendor. These shall be kept at a place as agreed to between the two.

J-4 NUMBER OF TESTS

J-4.1 Tests for the moisture, ash, total reducing sugars and Fiehe's test shall be conducted on each of the samples constituting a set of individual samples.

J-4.2 Test for specific gravity, sucrose percent, fructose-glucose ratio and acidity shall be conducted on the composite sample.

J-4.2.1 If Fiehe's test is positive, determination of hydroxymethyl furfural (HMF) content shall be carried out on the individual sample.

J-5 CRITERIA FOR CONFORMITY

J-5.1 A lot shall be declared to have satisfied the requirements of the specification when **J-5.1.1** and **J-5.1.4** are satisfied.

J-5.1.1 Each individual sample shall satisfy the requirements given in **5.1**, **5.2** and **5.3**.

J-5.1.2 The test results on the composite sample for characteristics mentioned in **J-4.2** and **J-4.2.1** shall satisfy the corresponding requirements as given in Table 1.

J-5.1.3 The test result on individual samples for Fiehe's test shall be negative. The HMF content determination, carried out on those individual samples in which Fiehe's test is positive, shall satisfy the corresponding requirements as given in Table 1.

J-5.1.4 The test results for moisture, ash and total reducing sugars shall be recorded as shown in Table 4. The mean and range for the test results of the individual sample shall be calculated as follows:

$$\text{Mean } (\bar{X}) = \frac{\text{sum of test results}}{\text{number of test results}}$$

Range (R) = the difference between the maximum and minimum values of the test results

The mean and range shall be recorded as shown in col 3 and 4 of Table 4 respectively. The appropriate expression as shown in col 6 of Table 4 shall be calculated. If the value of these expressions satisfy the relevant conditions given in col 6 of Table 4, the lot shall be declared to have satisfied the requirements of moisture, ash and total reducing sugars.

Table 4 Criteria for Conformity
(*Clause* J-5.1.4)

Sl No.	Characteristic	Test Results 1, 2, ... n	Average	Range	Criterion for Conformity
(1)	(2)	(3)	(4)	(5)	(6)
i)	Moisture	—	$\bar{X}_1$	R_1	$\bar{X}_1 + 0.6\,R_1 \leq$ the value of that grade as specified in Table 1
ii)	Ash	—	$\bar{X}_2$	R_2	$\bar{X}_2 + 0.6\,R_2 \leq 0.5$
iii)	Total reducing sugars	—	$\bar{X}_3$	R_3	$\bar{X}_3 - 0.6\,R_3 \geq$ the value of that grade as specified in Table 1

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