Bangladesh Standard
Specification For
Honey
(Second Revision)

ICS 67.180.10

BANGLADESH STANDARDS AND TESTING INSTITUTION
MINISTRY OF INDUSTRIES
MAAN BHABAN, 116-A, TEJGAON INDUSTRIAL AREA
DHAKA-1208, BANGLADESH

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Foreword

This Bangladesh standard was adopted by the Bangladesh Standards and Testing Institution on 5 July 2022, after the draft finalized by the Fruits, Vegetables and their Derived Products Sectional Committee had been approved by the Agricultural and Food Products Divisional Committee.

This standard 'Honey' was first published in 1983 and subsequently revised in 2002. Later on, in 2007 the Codex Standard ‘Codex Stan 12-1981-Standard for Honey, Revision 2001’ was adopted as Bangladesh standard superseding 'BDS 1039:2002 Specification for Honey'. But according to the recent comments of researchers and processors, the adopted standard was difficult to comply with due to geographical feature, sources of honey, variety of honeybee species for local producing honey. In order to resolve the issue, the technical committee decided to readopt the previous standard i.e. BDS 1039 with a revision and also possible harmonization with ‘CXS 12-1981 Standard for Honey, Amended in 2019’.

This standard is subject to periodical reviews and amendments, if necessary, in order to keep pace with the latest industrial and technological innovations. Any suggestions for improvement will be recorded and placed before the committee 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 places retained in the rounded off value should be the same as that of the specified value in the standard.

This standard cancels and replaces BDS CAC 12:2007 Specification for honey, which has been technically revised.

Introduction

0.1 Honey is a sweet, viscous food substance made by honey bees and some other bees. Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew), by regurgitation, enzymatic activity, and water evaporation. Honey bees store honey in wax structures called honeycombs, whereas stingless bees store honey in pots made of wax and resin. The variety of honey produced by honey bees (the genus *Apis*) is the best known, due to its worldwide commercial production and human consumption. Honey is collected from wild bee colonies, or from hives of domesticated bees, a practice known as beekeeping or apiculture.

0.2 In this document, the following verbal forms are used:

— “shall” indicates a requirement;
— “should” indicates a recommendation;
— “may” indicates a permission;
— “can” indicates a possibility or a capability.
— “NOTES” provide guidance in understanding or clarifying the requirements in this document.

Bangladesh Standard
Specification For
Honey
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1. Scope

This standard specifies the requirements, methods of sampling and test for honey.

2. Normative References

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. Definition

3.1 Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature.

3.2 Honey consists essentially of different sugars, predominantly fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection. The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallised. The flavour and aroma vary, but are derived from the plant origin.

4 Essential composition and quality factors

4.1 Honey shall be well-ripened natural product. Honey sold as such shall not have added to it any food ingredient, nor shall any other additions be made other than honey. It shall not have any objectionable matter, flavour, aroma, or taint absorbed from foreign matter during its processing and storage. It shall have been strained clear through a double layered cloth (150 microns) at a temperature not exceeding 60ºC. It shall not have begun to ferment or effervesce.

4.2 Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality is impaired.

4.3 Chemical or biochemical treatments shall not be used to influence honey crystallisation.

4.4 The honey shall also comply with the requirements given in Table 1.

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

Table-1 Requirements for Honey
(Clause 4.4)

Sl No	Characteristic	Limit	Method of Test
(1)	(2)	(3)	(4)
i)	Specific gravity at 27ºC, Min.	1.35	Annex B
ii)	Moisture, percent by mass, Max.	23.0[(1)]	Annex C
iii)	Fructose-glucose ratio[(2)]	0.95-1.45	Annex D
iv)	Total reducing sugar, percent by mass, Min.	65.0	Annex D
v)	Sucrose, percent by mass, Max.	5	Annex D
vi)	Ash, percent by mass, Max.	0.5	Annex E
vii)	Acidity (expressed as formic acid), percent by mass, Max.	0.2	Annex F
viii)	Fieh's test	Negative	Annex G
ix)	Hydroxymethyl furfural (HMF), mg/kg, Max.	80	Annex G
x)	Total count of pollens and plants elements/g of honey, Min.	5 000	Annex H
xi)	Optical density, at 660 nm, percent, Max.	0.3	Annex I
xii)	Diastase activity, Schade units, Min.	3	AOAC 958.09

[(1)] For Sundarbon's honey/raw honey (without further processing which permitted in section 4.1)/comb honey (see Note-5)/cut comb in honey or chunk honey (see Note-5), the maximum limit for moisture is 25 %

[(2)] If Fiehe's test is positive, carry out the determination of hydroxymethyl furfural (HMF) content. If it is more than 80 mg/kg, then fructose-glucose ratio should be more than 0.95

5. Packaging and Marking

5.1 **Packing** – 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 non-corrosive and non-reactive material to honey and shall be provided with cork washers to avoid spilling.

5.2 **Marking** – Each container shall be legibly and indelibly marked with the following information:

a) Name of the product shall be designated "Honey" (see Note-1, 2, 3, 4 and 5);
b) Name and address of the packer/importer;
c) Batch or code number;
d) Date of packing;
e) Best before on……;
f) Net quantity;
g) Maximum Retail Price (MRP);
h) Storage instruction;
l) Any other requirements as specified under the Bangladesh standards of weights and measures (packaged commodities) Rules, 2021.

Note-1 Honey may be designated by the name of the geographical or topographical region if the honey was produced exclusively within the area referred to in the designation.

Note-2 Honey may be designated according to floral or plant source if it comes wholly or mainly from that particular source and has the organoleptic, physicochemical and microscopic properties corresponding with that origin.

Note-3 When honey has been designated according to presence of specific pollen of floral or plant source then the common name or the botanical name of the floral source shall be in close proximity to the word "honey".

Note-4 Honey may be designated according to the method of removal from the comb.

(a) Extracted Honey is honey obtained by centrifuging decapped broodless combs.
(b) Pressed Honey is honey obtained by pressing broodless combs.
(c) Drained Honey is honey obtained by draining decapped broodless combs.

Note-5 Honey may be designated according to the following styles:

(a) Honey which is honey in liquid or crystalline state or a mixture of the two;
(b) Comb Honey which is honey stored by bees in the cells of freshly built broodless combs and which is sold in sealed whole combs or sections of such combs;
(c) Cut comb in honey or chunk honey which is honey containing one or more pieces of comb honey.

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

Note-6 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. Sampling

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 appropriate Annexes specified in col. 4 of Table 1.

7.2 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-7 Pure chemicals' shall mean chemicals that do not contain impurities, which may affect the result of analysis.

8. Compliance

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)
List of Relevant Standards

BDS	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

Annex - B
[Table-1, Item-(i)]
Determination of Specific Gravity

B.1 Apparatus

B.1.1 Thermostatically Controlled Water-Bath - Maintained at 27 ± 1 ºC.

B.1.2 Specific Gravity Bottle

B.2 Procedure

B.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.

B.3 Calculation

B.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.

B.4 Hydrometer Method

B.4.1 Apparatus – A hydrometer is an instrument used to determine specific gravity instantly. A hydrometer is made up of a thin glass or plastic tube sealed at both ends with a graduated or printed scale calibrated to a specific gravity. It operates based on the Archimedes principle that a solid body displaces its own weight within a liquid in which it floats.

B.4.2 Procedure - A glass or plastic cylinder, commonly known as a hydrometer jar, is filled with the honey sample being measured. [Proper Immersion: A hydrometer jar should have an inside diameter of approximately 1 inch (25mm) greater than the outside diameter of the hydrometer]. The hydrometer is then placed in the hydrometer jar containing the honey sample. Once the hydrometer has settled, take the reading from the scale. The specific gravity of the sample honey is indicated when the level of the sample liquid in the jar aligns with a point on the hydrometer scale. In order for the measurement to be accurate, the sample liquid must be at 60°F.

Annex - C
[Table-1, Item-(ii)]
Determination of Moisture

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.

C.1 Oven Drying Method

C.1.1 Apparatus

C.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.

C.1.1.2 Sand

Passing through 500-micron BDS Sieve but retained on 180-micron BDS 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.

C.1.1.3 Vacuum Oven

C.1.2 Procedure

C.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.

C.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^{o}C and 70^{o}C and at a pressure not more than 50 mm of mercury.

C.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 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 weightings do not differ by more than 0.5 mg.

C.1.3 Calculation

C-1.3.1 Moisture, percent by mass = $\frac{100\ (M_1 - M_2)}{M_1 - M}$

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.

C.2 Refractometer Method

C.2.1 Apparatus

C.2.1.1 Refractometer

C.2.2 Procedure - Determine the refractometer reading of honey at 20^{o}C and calculate the percentage of moisture from the values given in Table 2. If the determination is made at a temperature other than 20ºC, 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
1499 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
1491 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 ºC, add the correction; if made below, subtract the correction.

Annex - D

[Table-1, Item-(iii), (iv) and (v)]

Determination of Total Reducing Sugars, Sucrose and Fructose-Glucose Ratio

D.1 Total Reducing Sugars

D.1.1 Reagents

D.1.1.1 Soxhlet Modification of Fehling's Solution

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

D.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.

D.1.1.2.1 Standardization of copper sulphate solution

Using separate pipettes, pipette out accurately 5 ml of Solution A (D-1.1.2) and 5 ml of Solution B (D-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 (D-1.1.5) from a burette, about one millitare 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 5ml of copper sulphate solution.

D.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.

D.1.1.4 Hydrochloric Acid

Sp gr 1.18 at 20^{o}C (approximately 12 N).

D.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 1000 ml; 50 ml of this solution contains 0.05 g invert sugar.

D.1.1.6 Methylene Blue Indicator - 0.2 percent in water.

D.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 (D-1.1.2) and Solution B (D-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.

D.1.3 Calculation

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.

D.2 Sucrose

D.2.1 Procedure

To 100 ml of the stock honey solution (see D-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 D-1.2.

D.2.2 Calculation

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

D.3 Fructose-Glucose Ratio

D.3.1 Reagents

D.3.1.1 Iodine Solution - 0.05 N.

D.3.1.2 Sodium Hydroxide Solution - 0.1 N.

D.3.1.3 Sulphuric Acid - concentrated.

D.3.1.4 Standard Sodium Thiosulphate Solution - 0.05 N.

D.3.2 Procedure

Pipette 50 ml of honey solution (see D-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.

D.3.3 Calculation

D.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.

D.3.3.2 Approximate fructose, percent by mass (x) =

$$\frac{Approximate\ total\ reducing\ sugars,\ percent - w}{0.925}$$

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

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

True reducing sugars, percent by mass = y + z

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

Annex - E
[Table-1, Item-(vi)]
Determination of Ash

E.1 Procedure

E.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 ^{o}C till white ash is obtained. Cool the dish in a desiccators and weigh. Incinerate to constant weight.

E.2 Calculation

E.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 - F
[*Table-1, Item*-(vii)]
Determination of Acidity

F.1 Reagents

F.1.1 Standard Sodium Hydroxide Solution - 0.05 N.

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

F.2 Procedure

F.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.

F.3 Calculation

F.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 - G
[*Table-1, Item* (viii) *and* (ix)]
Fiehe's Tests and Determination of Hydroxymethyl Furfural (HMF) Content

Carry out the hydroxymethyl furfural (HMF) test (G-2) only when Fiche's test (G-1) is positive.

G.1 Fiehe's Test

G.1.1 Reagents

G.1.1.1 Resorcinol Solution- Dissolve 1 g resublimed resorcinol in 100 ml hydrochloric acid (sp gr 1.18 to 1.19).

G.1.1.2 Ether - sulphuric ether.

G.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.

G.2 Determination of HMF Content

G.2.1 Reagents

G.2.2.2 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, and make up to volume.

G.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 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.

G.2.2 Distilled Water (Oxygen Free)

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

G.2.3 Apparatus

G.2.3.1 Spectrophotometer to Read at 550 nm

G.2.3.2 Water Bath

G.2.4 Sampling

G.2.4.1 The honey is prepared as in 6.1 without any heating.

G.2.5 Procedure

G.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.

G.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 mm using a 1 cm cell, immediately after the maximum value is reached.

G.2.6 Calculation

G.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 spectro-photometrically where € = 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. Results are expressed as mg HMF/kg honey.

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

Annex - H
[*Table-1, Item*-(x)]
Determination of Total Count of Pollens And Plant Elements in Honey

H.1 Procedure

H.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 3000 rev/min for 5 minutes. Decant cautiously the supernatant liquid without disturbing the sediment, taking care to leave one milliliter 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 milliliter 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 X. Repeat this counting ten times and take 10 different counts with the dispersed sediment.

H.2 Calculation

H.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-I
[*Table-1, Item*-(xi)]
Determination of Optical Density of Honey

I.1 Procedure

I.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 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 " 150	4	6
151 " 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 being the total number of containers in the lot, n the number of containers to be chosen (see Table 3). Every r^{th} 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 approximately 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 Fiche'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 4.1, 4.2 and 4.3.

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:

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

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	-	$\ddot{X}_1$	R_1	$\ddot{X}_1 + 0.6\ R_1 \leq$ the value of that grade as specified in Table 1
ii)	Ash	-	$\ddot{X}_2$	R_2	$\ddot{X}_2 + 0.6\ R_2 \leq 0.5$
iii)	Total reducing sugars	-	$\ddot{X}_3$	R_3	$\ddot{X}_3 - 0.6\ R_3 \geq$ the value of that grade as specified in Table 1

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