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SPECIFICATION FOR
HONEY
(First Revision)

SRI LANKA STANDARDS INSTITUTION

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SLS 464 : 2016

Gr. 11

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Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This standard does not purport to include all the necessary provisions of a contract.

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FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2016-10-06.

Honey is naturally obtained mainly from *Apis cerana indica*, *Apis mellifera* and *Apis dorsata* bees.

This Standard was first published in 1979. It was felt necessary to revise this standard because of the improvement of current trade practices in this country since the last publication and due to increasing consumer awareness of quality products. While preparing this standard, the increasing consumer demand of forest and apiary honey was considered. In this revision the title of the standard has been changed by removing the word "Bees". New grade has been introduced as "Dorsata" in addition to the special and standard honey grades. Sensory characteristics and microscopic examinations have been included to the requirements clause.

This Standard is subjected to the restrictions imposed under the Sri Lanka Food Act No. 26 of 1980 and the regulations framed thereunder.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the results of a test or analysis shall be rounded off in accordance with **SLS 102**. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

In the revision of this Standard, the assistance obtained from the publications of Bureau of Indian Standards, Codex Alimentarius Commission and the European Honey Commission are gratefully acknowledged.

1 SCOPE

1.1 This Standard prescribes the requirements and methods of sampling and test for honey.

2 RFERENCES

Official Methods of Analysis, Association of Official Analytical Chemists (AOAC) 18th edition, 2007

SLS 102 Rules for rounding off numerical values

SLS 143 Recommended code of practice for general principles of food hygiene
SLS 428 Random sampling methods
SLS 467 Code of practice for labeling of pre packaged foods

3 DEFINITIONS

For the purpose of this Standard the following definition shall apply:

3.1 honey: 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

4 GRADES

Honey shall be of three grades namely, Special, Standard and Dorsata.

4.1 Special grade

It shall be the well ripened, natural product produced by honey bees (*Apis cerana indica* and *Apis mellifera)* in domesticated hives. It can be in the liquid or partially crystallized form. In the liquid form it shall be clear and visually transparent. It shall be free from objectionable flavours and odours. In the preparation of special grade honey for marketing it shall not be heated above 45 ^{o}C. It shall be free from signs of fermentation or be effervescent.

4.2 Standard grade

It shall be the well ripened, natural product produced by honey bees *(Apis cerana indica* and *Apis mellifera)* at a natural habitat and can be in the liquid or partially crystallized form. It shall be free from objectionable flavours and odours. In the preparation of standard grade honey for marketing it shall not be heated above 45 ^{o}C. It shall be free from signs of fermentation or be effervescent.

4.3 Dorsata grade

It shall be the well ripened, natural product produced by *Apis dorsata.* It shall be a light viscous solution with characteristic flavour. In the preparation of dorsata grade honey for marketing it shall not be heated above 45 ^{o}C. It may show signs of fermentation with prolonged storage.

5 REQUIREMENTS

5.1 Hygiene

The product shall be processed, packaged, stored and distributed under hygienic conditions as prescribed in **SLS 143**.

5.2 Additives

Honey shall not have any added food ingredient, including food additives.

5.3 Foreign matter

Honey shall be free from foreign matter such as moulds, insects, insect debris, brood or sand. Honey shall not have any objectionable matter, flavour, aroma or taint absorbed from foreign matter during its processing and storage.

5.4 Adulterants

Honey shall be free from adulterants including all plant parts except pollen grains and it may contain bee appendages as illustrated in Figure **1** and **2** when examined in accordance with the method prescribed in Appendix **J**.

5.5 Contaminants

Residues of antibiotics, pesticides and veterinary drugs shall not be permitted in honey.

5.6 Other requirements

Honey shall comply with the requirements given in Tables **1**, **2** and **3**.

5.6.1 *Sensory characteristics*

TABLE 1 – Sensory characteristics of honey

Sl No (1)	**Characteristic** (2)	**Standard grade** (3)	**Special grade** (4)	**Dorsata grade** (5)
i)	Flavour	Characteristic	Characteristic	Characteristic
ii)	Odour	Characteristic	Characteristic	Characteristic
iii)	Colour	Pale yellow to dark amber	Pale yellow to dark amber	Pale yellow to amber
iv)	Consistency	Partially crystallized/ Viscous	Partially crystallized/ Viscous	Fluid/ Light viscous

5.6.2 *Chemical requirements*

The product shall conform to the requirements given in Table **2** when tested in accordance with the methods prescribed ion Column **6** of the table.

TABLE 2 – Chemical requirements for honey

Sl No. (1)	**Characteristic** (2)	**Grade**			**Method of test** (6)
		Special (3)	**Standard** (4)	**Dorsata** (5)	
i)	Moisture percent by mass, max.	20	22	25	Appendix **B**
ii)	Total reducing sugars percent by mass, min.	68	65	62	Appendix **C**
iii)	Sucrose percent by mass, max.	5	5	1	Appendix **D**
iv)	Ash percent by mass, max.	0.5	0.5	0.5	Appendix **E**
v)	Acidity expressed as formic acid, percent by mass, max.	0.1	0.1	0.1	Appendix **F**
vi)	Hydroxy methyl furfural (HMF) content mg/ kg max **(*NOTE)**	40	50	30	Appendix **G**
vii)	Fructose glucose ratio, min.	0.95	0.95	0.80	Appendix **H**

***NOTE:**

*Carry out the determination of hydroxyl methyl furfural content, only when Fiehe's test is positive (Appendix **G.2**).*

5.6.3 *Heavy metals*

The product shall conform to the tolerance limits for heavy metals given in Table **3** when tested in accordance with the methods prescribed in Column **4** of the table.

TABLE 3 – Limits for heavy metals

Sl No (1)	**Heavy metal** (2)	**Limit** (3)	**Method of test** (4)
i)	Arsenic as As, mg/ kg, max	0.1	**AOAC 986.15**
ii)	Lead as Pb, mg/ kg, max	0.1	**AOAC 999.10**
iii)	Cadmium as Cd, mg/ kg, max	0.1	**AOAC 999.10**

6 PACKAGING

The honey shall be packaged in hygienically clean, transparent, wide mouthed, well sealed glass or any other suitable food grade containers which are acid resistant and non–reactive to the contents.

7 MARKING AND/ OR LABELING

7.1 The following shall be marked or labeled legibly and indelibly on each package/ container destined to the final consumer:

a) Name of the product as "Bees Honey (මී පැණි)" or "Dorsata Honey (බඹර පැණි)" and grade when applicable;
b) Brand name or trade mark, if any;
c) Net content in "g", "kg", "ml" or "l";
d) Instructions for storage and use, if any;
e) Batch number or code number or a decipherable code marking;
f) Name and address of the processor and packer/ distributor in Sri Lanka;
g) Country of origin;
h) Date of processing;
j) Date of packaging/ bottling; and
k) Date of expiry.

7.2 The marking and labeling shall also be in accordance with **SLS 467**.

8 SAMPLING

Representative samples of honey shall be drawn according to the method prescribed in Appendix **A.**

9 METHODS OF TEST

Tests shall be carried out as given in Appendices **B** to **J** of this Standard.

10 CRITERIA FOR CONFORMITY

A lot shall be declared as conforming to the requirements of this Standard if the following conditions are satisfied:

10.1 Each container examined as in **A.4.1** satisfies the packaging and marking and/ or labeling requirements of this Standard.

10.2 Each container examined as in **A.4.2** satisfies the requirements given in clause **5.2**, **5.3**, **5.4** and **5.6.1**.

10.3 The test results of the composite sample when tested as in **A.4.3** satisfy the relevant requirements given in clause **5.5. 5.6.2** and **5.6.3**.

APPENDIX A
SAMPLING

A.1 LOT

In any consignment, all the containers of the same size and belonging to one batch of manufacture or supply shall constitute a lot.

A.2 GENERAL REQUIREMENTS OF SAMPLING

In drawing, preparing, sorting and handling samples, following precautions and directions shall be taken:

A.2.1 Samples shall be drawn in a protected place not exposed to damp air, dust or soot.

A.2.2 The sampling instruments shall be clean and dry when used.

A.2.3 The samples shall be placed in clean and dry glass containers. The size of the sample containers shall be of such that they are almost completely filled by the sample.

A.2.4 Precautions shall be taken to protect the samples, the material being sampled, the sampling instruments and the sample container from adventitious contamination.

A.2.5 The sample containers shall be sealed air-tight after filling and marked with the necessary details of sampling.

A.2.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the room temperature.

A.2.7 When taking samples for microscopic examination in addition to the requirements given in **A.2.1** to **A.2.6,** the following precautions shall be observed:

A.2.7.1 The sampling instruments and the sample containers shall be sterile when used.

A.2.7.2 Examinations shall be carried out immediately after sampling.

A.3 SCALE OF SAMPLING

A.3.1 Samples shall be tested from each lot for ascertaining its conformity to the requirements of this specification.

A.3.2 The number of containers to be selected from a lot shall be in accordance with Column **2** of Table **4.**

Table 4 – Scale of sampling

Number of containers in the lot (1)	**Number of containers to be selected** (2)
Up to 25	6
26 to 150	9
151 to 500	12
501 and above	15

A.3.3 If the containers are packaged in cases at least 10 per cent of the cases subject to a minimum of two shall be selected from the lot. As far as possible an equal number of containers shall be drawn from each case so as to form a sample as given in Column **2** of Table **4**.

A.3.4 The cases and containers shall be selected at random. In order to ensure randomness of selection, random number tables as given in **SLS 428** shall be used.

A.3.5 The containers selected shall be marked with necessary details of sampling.

NOTE:
In case of quantity of material selected for testing of requirements is insufficient (sachet type packages), required number of samples shall be drawn from the lot.

A.3.6 Reference samples

If a reference sample is required, the number of containers to be selected from a lot shall be three times the number given in Column **2** of Table **4**. The containers so selected shall be divided into three equal parts. One of these parts shall be marked for the purchaser, one of the supplier and the third for the referee.

A.4 NUMBER OF TESTS

A.4.1 Each container selected as in **A.3.2** or **A.3.3** shall be examined for packaging and marking and/ or labeling requirements of this standard.

A.4.2 Each of the containers selected as in **A.3.2** or **A.3.3** shall be individually tested for the requirements given in Clause **5.2, 5.3, 5.4** and **5.6.1**.

A.4.3 After testing for requirements as stated in **A.4.2** equal quantities of material shall be taken from each container and mixed together to form a composite sample. The composite sample thus obtained shall be tested for the requirements given in Clause **5.4, 5.6.2** and **5.6.3**.

APPENDIX B
DETERMINATION OF MOISTURE CONTENT

B.1 APPARATUS

B.1.1 *Refractometer*

B.2 PROCEDURE

Prepare the honey for testing as in **C.3**.

B.2.1 Determination of the refractive index

Determine the refractive index of the test sample using a refractometer at a constant temperature near 20 ^{o}C, according to the temperature corrections indicated in Table **5**. The method used is to be noted in test report.

B.2.2 Temperature corrections

Temperature above 20 ^{o}C: Add 0.000 23 per 1 centigrade
Temperature below 20 ^{o}C: Subtract 0.000 23 per 1 centigrade

TABLE 5 – Estimation of moisture content

Refractive Index (20 ^{o}C)	**Moisture content (per cent by mass)**	**Refractive Index (20 ^{o}C)**	**Moisture content (per cent by mass)**	**Refractive Index (20 ^{o}C)**	**Moisture content (per cent by mass)**
(1)	(2)	(3)	(4)	(5)	(6)
1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.6
1.5033	13.4	1.4925	17.6	1.4820	21.8
1.5028	13.6	1.4920	17.8	1.4815	22.0
1.5023	13.8	1.4915	18.0	1.4810	22.2
1.5018	14.0	1.4910	18.2	1.4805	22.4
1.5012	14.2	1.4905	18.4	1.4800	22.6
1.5007	14.4	1.4900	18.6	1.4795	22.8
1.5002	14.6	1.4895	18.8	1.4790	23.0
1.4997	14.8	1.4890	19.0	1.4785	23.2
1.4992	15.0	1.4885	19.2	1.4780	23.4
1.4987	15.2	1.4880	19.4	1.4775	23.6
1.4982	15.4	1.4875	19.6	1.4770	23.8
1.4976	15.6	1.4870	19.8	1.4765	24.0
1.4971	15.8	1.4865	20.0	1.4760	24.2
1.4966	16.0	1.4860	20.2	1.4755	24.4
1.4961	16.2	1.4855	20.4	1.4750	24.6
1.4956	16.4	1.4850	20.6	1.4745	24.8
1.4951	16.6	1.4845	20.8	1.4740	25.0
1.4946	16.8	1.4840	21.0		
1.4940	17.0	1.4835	21.2		

APPENDIX C
DETERMINATION OF REDUCING SUGAR CONTENT

C.1 PRINCIPLE OF THE METHOD

The method involves reduction of Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator. The maximum accuracy for this type of determination is attained by ensuring that the reduction of the Fehling's solution during the standardization step and in the determination of the reducing sugars in the honey solution are carried out at constant volume. A preliminary titration is, therefore, essential to determine the volume of water to be added before the determinations are carried out to satisfy this requirement.

C.2 REAGENTS

C. 2.1 Soxhlet's modification of Fehling's solution

Solution A: Dissolve 69.28 g of Copper sulphate pentahydrate ($CuSO_4.5H_2O$; mol. wt: 249.71) in distilled water and dilute to one litre. Keep for a day before titration.

Solution B: Dissolve 346 g of Sodium potassium tartrate ($C_4H_4K\ NaO_6.4H_2O$; mol. wt: 282.23) and 100 g of Sodium hydroxide (NaOH) in distilled water and dilute to one litre. Filter through prepared asbestos.

C.2.2 Standard invert sugar solution (10 g/ l)

Weigh, accurately to the nearest milligram, 9.5 g of pure sucrose, add 5 ml Hydrochloric acid (approximately 36.5 per cent by mass, pure HCl) and dilute with water to about 100 ml. Store this acidified solution for several days at room temperature, and then dilute to one litre. Neutralize a suitable volume of this solution with 1 mol/ l Sodium hydroxide solution (40 g/ l) immediately before use and dilute to the required concentration (2 g/ l) for the standardization.

NOTE:
Acidified 1.0 per cent invert sugar remains stable for several months

C.2.3 Methylene blue solution

Dissolve 2 g of Methylene blue in distilled water and dilute to one litre.

C.2.4 Alumina cream

Prepare a cold saturated solution of alum ($K_2SO_4.AI_2(SO_4)_3.24H_2O$) in water. Add Ammonium hydroxide with constant stirring until solution is alkaline to litmus. Let precipitate settle and wash by decantation with water until wash-water gives only slight

turbidity for sulphate with Barium chloride solution. Pour off excess water and store residual cream in stoppered bottle.

C.3 PROCEDURE

C. 3.1 Preliminary treatment of sample

C.3.1.1 *Liquid or strained honey*

If the sample is free from granulation, mix thoroughly by stirring or shaking, if granulated, place closed container in water bath without submerging, and heat for 30 minutes at 60 ^{o}C; then if necessary heat at 65 ^{o}C until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as sample liquefies. Do not heat honey intended for Hydroxy methyl furfural or diastatic determination. If foreign matter (eg: wax, sticks, bees, particles of comb) is present, heat the sample to 40 ^{o}C in a water bath and strain through cheesecloth in hot water funnel before sampling.

C.3.1.2 *Comb honey*

Cut across top of comb if sealed, and separate completely from comb by straining through a sieve, the meshes of which are made by weaving wire so as to from a square opening of 0.500 mm. When portions of comb or wax pass through sieve, heat the sample as in **C.3.1.1** and strain through cheesecloth. If honey is granulated in comb, heat until wax is liquefied, stir, cool and remove wax.

C.3.2 Preparation of the test sample

Two methods are prescribed as follows.

C.3.2.1 *Method 1*

Applicable to honey which may contain sediment

C.3.2.1.1 Weigh accurately, to the nearest milligram, 25 g of the homogenized honey, transfer to a 100-ml volumetric flask, add 5 ml alumina cream (**C.2.4**), dilute to volume with distilled water at 20 ^{o}C and filter.

C.3.2.1.2 Dilute 10 ml of this solution up to 500 ml with distilled water (diluted honey solution).

C.3.2.2 *Method 2*

C.3.2.2.1 Weight accurately, to the nearest milligram 2 g of the homogeneous honey sample, dissolve in distilled water and dilute to 200 ml in a calibrated flask (honey solution).

C.3.2.2.2 Dilute 50 ml of the honey solution up to 100 ml using distilled water (diluted honey solution).

C.3.3 Standardization of the modified Fehling's solution

Standardize the Fehling's solutions, mixing 5.0 ml each of Fehling's solution A and B with 2 g/ l invert sugar solution using Methylene blue indicator.

C.3.4 Preliminary titration

Total volume of the added reactants at the completion of the reduction titration must be 35 ml. This is made up by the addition of a suitable volume of water before the titration commences. Since the compositional criteria of the honey Standard specify that there should be more than 60 per cent reducing sugars (calculated as invert sugar), a preliminary titration is necessary to establish the volume of water to be added to a given sample, to ensure the reduction is carried out at constant volume. This volume of water to be added is calculated by subtracting the volume of diluted honey solution consumed in the preliminary titration (*x* ml) from 25 ml. Pipette 5 ml Fehling's solution A in to a 250-ml Erlenmeyer flask and add approximately 5 ml of Fehling's B solution. Add 7 ml distilled water, a little powdered pumice or other suitable antibumping agent, followed by about 5 ml diluted honey solution from a burette. Heat the cold mixture until it boils over a wire gauze, and maintain moderate ebullition for two minutes. Add 1 ml of 0.2 per cent aqueous Methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 minutes, by repeated small additions of diluted honey solution until the indicator is decolorized. It is the colour of the supernatant liquid that must be observed. Note the total volume of diluted honey solution used (*x* ml).

C.3.5 Determination

C.3.5.1 Calculate the amount of added water necessary to bring the total volume of the reactants at the completion of the titration to 35 ml by subtracting the preliminary titration (*x* ml) from 25 ml.

C.3.5.2 Pipette 5 ml Fehling's solution A into a 250-ml Erlenmeyer flask and add approximately 5 ml Fehling's solution B.

C.3.5.3 Add (25 *x)* ml distilled water, a little powdered pumice or other suitable antibumping agent and from a burette, all about 1.5 ml of the diluted honey solution, volume determined in the preliminary titration. Heat the cold mixture to boiling over a wire gauze and maintain moderate ebullition for two minutes. Add 1 ml of 0.2 per cent methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 minutes by repeated small additions of diluted honey solution until the indicator is decolorized. Note the total volume of diluted honey solution *(y* ml). Duplicate titrations should agree within 0.1 ml.

C.3.5.4 It is essential to the accuracy and repeatability of the determination that the volume of water necessary to bring the reactant mixture to a total volume of 35 ml be determined for each individual sample. Table **6** gives typical volumes which may be encountered at the preliminary titration stage for the incremental contents of invert sugar shown, assuming the test sample **C.3.2.1** weighs about 25 g or test sample **C.3.2.2** weighs about 2 g.

Table 6 – Relevant volume of distilled water required for a given content of invert sugar

Invert sugar content (%) (1)	**Volume of distilled water to be added (ml)** (2)
60	8.3
65	9.6
70	10.7
75	11.6

C.4 CALCULATION

$$\textit{Grams invert sugar per 100 g honey (per cent)} = \frac{2000}{M \times Y}$$

where,

M is the mass of honey sample taken, in g; and
Y is the volume of diluted honey solution consumed in the determination, in ml.

APPENDIX D
DETERMINATION OF APPARENT SUCROSE CONTENT

D.1 REAGENTS

D.1.1 *Soxhlet modification of Fehling's solution* (see **C.2.1**)

D.1.2 *Standard invert sugar solution* (*see* **C.2.2**)

D.1.3 *Hydrochloric acid,* 5 mol/ l

D.1.4 *Sodium hydroxide solution,* 5 mol/ l

D.1.5 *Methylene blue solution,* 2 g/1 **(**see **C.2.3**)

D.2 PROCEDURE

Prepare the honey for testing as in **C.3**.

D.2.1 Preparation of test sample

Prepare the honey sample as in **C.3.2.1.1**. Dilute 10 ml of this solution to 250 ml with distilled water, diluted honey solution OR prepare the honey solution as in **C.3.2.2.1**.

D.2.2 Hydrolysis of the test sample

Place 50 ml of honey solution in a 100- ml graduated flask. Add 25 ml of distilled water into that and heat the test sample to 65 ^{o}C over a boiling water bath for 20 minutes (depending on the concentration). Remove the sample from the water bath and add 10 ml of conc HCl (5 mol/ l) to it. Immediately cool down the solution to the room temperature by dipping the sample in a cool water bath or holding the sample onto running tap water. Neutralize the sample with 5 mol/ l Sodium hydroxide, using litmus papers as the indicator. Adjust the volume of the sample to 100 ml (diluted honey solution). Titrate as in **C.3.4** and **C.3.5.**

D.3 CALCULATION

Calculate the percentage of invert sugar (grams of invert sugar per 100 g of honey) after inversion using the same formula as for per cent invert sugar before inversion in **C.4.**

Apparent sucrose content = (invert sugar content after inversion – invert sugar content before inversion) x 0.95

The result is expressed as grams apparent sucrose per 100 g of honey.

APPENDIX E
DETERMINATION OF MINERAL CONTENT (ASH)

E.1 PROCEDURE

Prepare the honey for testing as in Appendix **C.3**.

E.1.1 Ignition of honey

Weigh 5 g to 10 g of honey into an ignited and pre weighed platinum or silica dish. Weighing shall be carried out accurately to the nearest milligram. Heat gently in a muffle furnace until the sample is black and dry and there is no danger of loss by foaming and over flowing. An infra red lamp may also be used to char the sample before inserting into the furnace. If necessary a few drops of olive oil may be added to prevent frothing. Next, ignite the sample to constant mass at 600 ± 20 ^{o}C. Cool the residue (ash) in a desiccator and weigh. Express the results as per cent ash by mass.

APPENDIX F
DETERMINATION OF ACIDITY

F.1 REAGENTS

F.1.1 *Sodium hydroxide*, 0.1 mol/ l (carbonate-free)

F.1.2 *Distilled water*, made free of carbon dioxide by boiling and subsequent cooling

F.2 PROCEDURE

Prepare the honey for testing as in **C.3**.

F.2.1 Preparation of test sample

Weigh, accurately to the nearest milligram, 10.0 g of honey and dissolve in 75 ml distilled water, (see **F.1.2**).

F.2.2 Titration

Titrate the test sample against carbonate free 0.1 mol/ l Sodium hydroxide solution, using four to five drops of neutralized phenolphthalein indicator. The end point colour should persist for 10 seconds. Use a smaller mass, for darkly coloured samples. As an alternative, a pH meter may be used and the sample titrated to pH 8.3.

F.3 CALCULATION

$$\textit{Acidity (as formic acid, per cent by mass)} = \frac{V \times N \times 0.46}{M_1}$$

where,

V is the volume of the standard sodium hydroxide solution required for the titration in ml; and

M_1 is the mass of the honey taken for the test in g.

APPENDIX G
PHOTOMETRIC DETERMINATION OF HYDROXY METHYL FURFURAL (HMF) CONTENT

G.1 FIEHE'S TEST

G.1.1 Reagents

G.1.1.1 *Resorcinol solution*

Dissolve 1 g of resorcinol in 100 ml Hydrochloric acid (relative density 1.18 to 1.19).

G.2.1.2 *Filler*

Petroleum ether 40/ 60 ^{o}C

G.1.2 Procedure

Transfer about 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 indicate a negative reaction.

G.2 HYDROXY METHYL FURFURAL (HMF) CONTENT

G.2.1 Apparatus

G.2.1.1 *Spectrophotometer*

G.2.1.2 *Water bath*

G.2.2 Reagents

G.2.2.1 *Barbituric acid solution*

Weigh 500 mg barbituric acid and transfer to a 100-ml graduated flask using 60 ml water. Place in a hot water bath until dissolved, cool and make up to volume.

G.2.2.2 *P- toluidine solution*

G.2.2.3 *Distilled water (oxygen free)*

Nitrogen gas is passed through boiling distilled water, the water is then cooled.

G.2.3 Procedure

The honey is prepared for testing as in **C.3** without any heating.

G.2.3.1 *Preparation of test sample*

Weigh 10 g of honey sample and dissolve without heating in 20 ml oxygen-free distilled water (see **G.2.1.3**). Transfer to a 50-ml graduated flask and made up to volume (honey solution). Test after preparation without delay.

G.2.3.2 *Photometric determinations*

Pipette 2.0 ml of honey into two test tubes and add 5.0 ml of p-toluidine solution to each tube. Add 1 ml of water to one test tube and 1 ml of barbituric acid solution to the other test

tube. Shake the tubes well. Tube with added water will serve as the water blank. Read the absorbance of the sample against the blank at 550 nm using a 1-cm cell, immediately when the maximum value is reached. Read the concentration of HMF from the standard curve.

NOTE:
The addition of the reagents should be done without pause and should be finished in about one to two minutes.

G.2.4 Standard curve and expression of results

Draw the standard curve showing HMF concentration against absorbance, using 0 µg to 300 µg standard solution of HMF. Arrange HMF spectrometrically at 289 nm (€ = 16,830) before use.

Concentration of HMF can be obtained from the equation :

$$HMF \quad (mg/100\,g) = \frac{Absorbance \quad x \quad 19.2}{Thickness \quad of \quad layer}$$

Results are expressed as mg HMF/ kg honey.

APPENDIX H
DETERMINATION OF FRUCTOSE – GLUCOSE RATIO

H.1 REAGENTS

H.1.1 *Iodine solution,* 0.025 mol/ l

H.1.2 *Sodium hydroxide solution,* 0.1 mol/ l

H.1.3 *Sulphuric acid*, concentrated

H.1.4 *Sodium thiosulphate solution,* 0.05 mol/ l

H.2 PROCEDURE

Weigh, to the nearest milligram, 1 g of the prepared honey (**C.3**) into a 250-ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly and make the volume to 250 ml with water. Pipette 50 ml of this honey solution into a 250-ml stoppered flask. Add 40 ml of iodine solution and 25 ml of Sodium hydroxide solution. Stopper the flask and keep in the dark for 20 minutes. Acidify with 5 ml of concentrated Sulphuric acid and titrate quickly the excess of iodine against standardized Sodium thiosulphate solution. Conduct a blank using 50 ml of water instead of honey solution.

H.3 CALCULATIONS

H.3.1

$$\text{Glucose, per cent by mass} = \frac{(B - S) \times 0.004\,302}{M_2} \times 100$$

where,

B is the volume of 0.05 mol/ l Sodium thiosulphate solution required for the blank in ml;

S is the volume of 0.05 mol/ l Sodium thiosulphate solution required for the sample in ml; and

M_2 is the mass of 50 ml of the honey solution in g.

H.3.2 *Fructose, percent by mass = total reducing sugars, per cent by mass – glucose, per cent by mass*

H.3.3 $\text{Fructose – glucose ratio} = \frac{\text{fructose, per cent by mass (H.3.2)}}{\text{Glucose, per cent by mass (H.3.1)}}$

APPENDIX J
MICROSCOPIC EXAMINATION

J.1 APPARATUS

J.1.1 *Microscope*

J.1.2 *Microscope slides*

J.1.3 *Cover slips*

J.1.4 *Glass rod*

J.2 REAGENTS

Freshly prepared 0.1 per cent Iodine solution

J.3 PROCEDURE

Place 1 or 2 drops of honey on a clean glass slide with the help of a clean glass rod and then place a clean glass cover slip to make a uniform distribution throughout the cover slip without making air bubbles between the cover slip and the slide. Observe under 10X of the microscope.

In order to observe starch grains, mix a drop of freshly prepared Iodine solution and a drop of honey and observe under 100X of the microscope.

Examine at least 5 slides prepared as above under the microscope and observe for the common adulterants which cannot be present in natural honey (Figure 1(a), 1(b), 1(c), 1(d), 1(e), 1(f)) and plant & animal parts which can be present in natural honey (Figure 2(a), 2(b)).

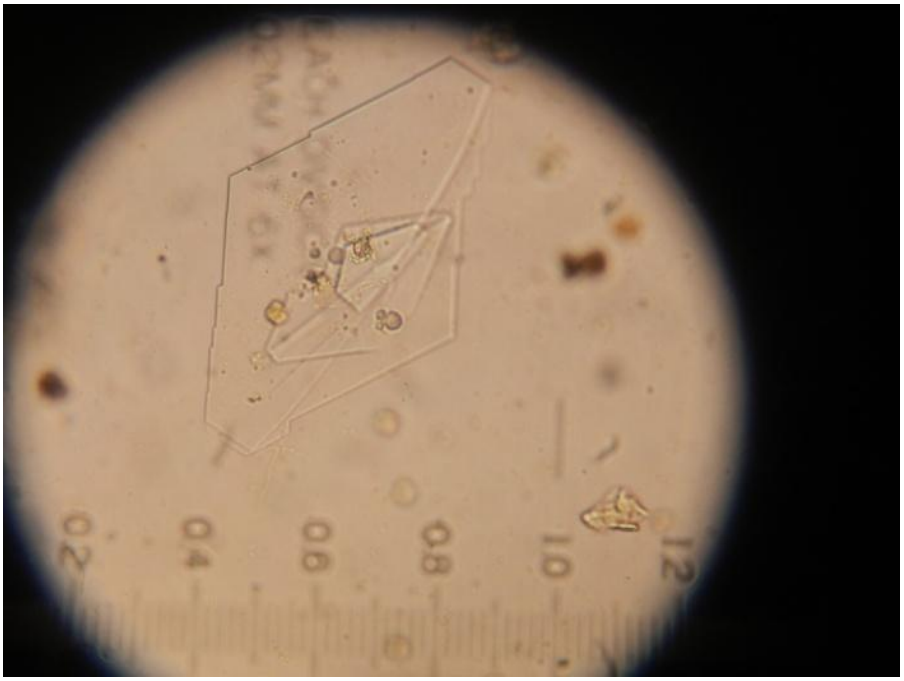

Figure 1(a): A photomicrograph of sugar crystals (100X)

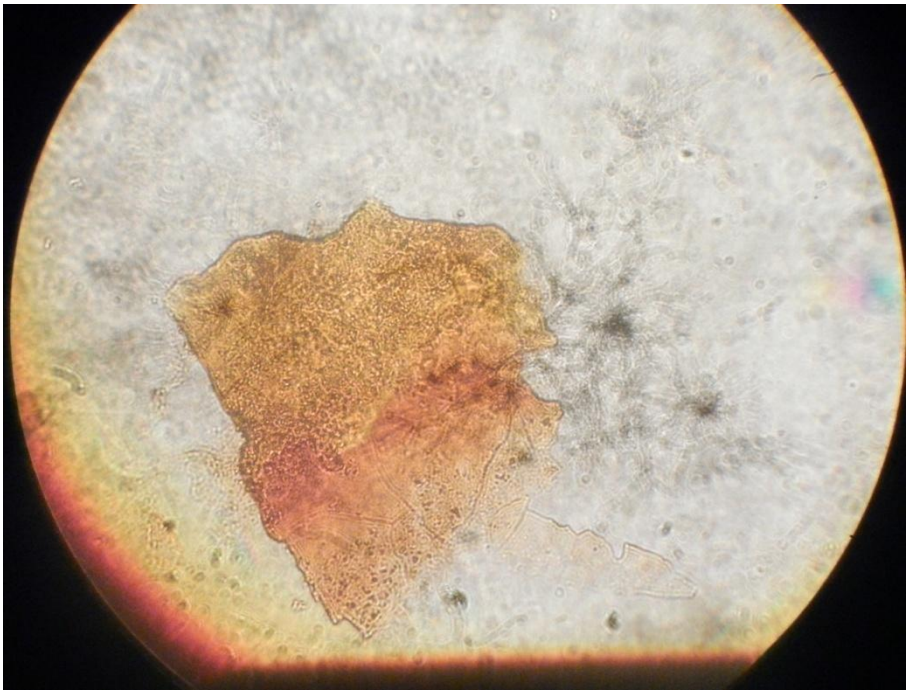

Figure 1(b): A photomicrograph of flower petal (100X)

Figure 1(c): A photomicrograph of fiber from the flower inflorescence (100X)

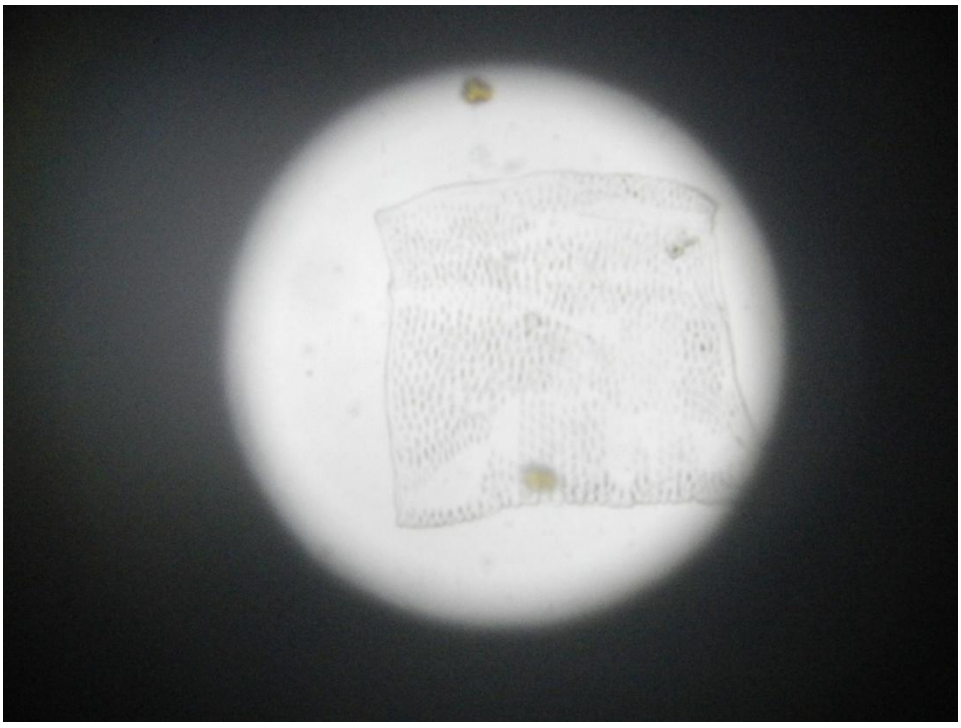

Figure 1(d): A photomicrograph of sclerenchyma of the flower (100X)

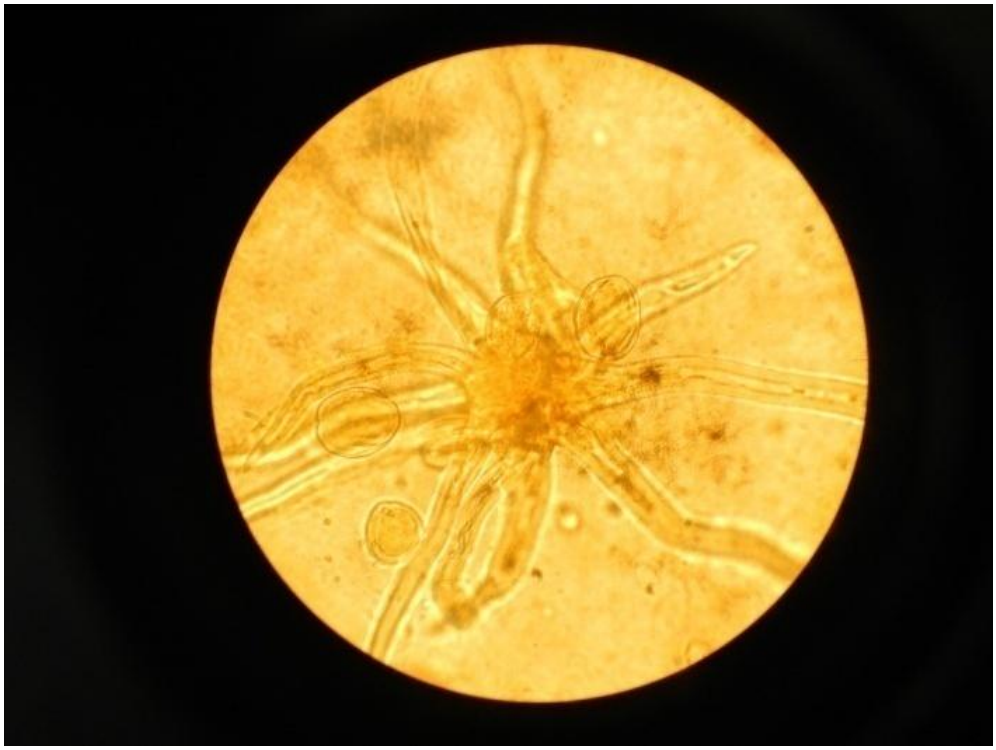

Figure 1(e): A photomicrograph of trichome of a flower (100X)

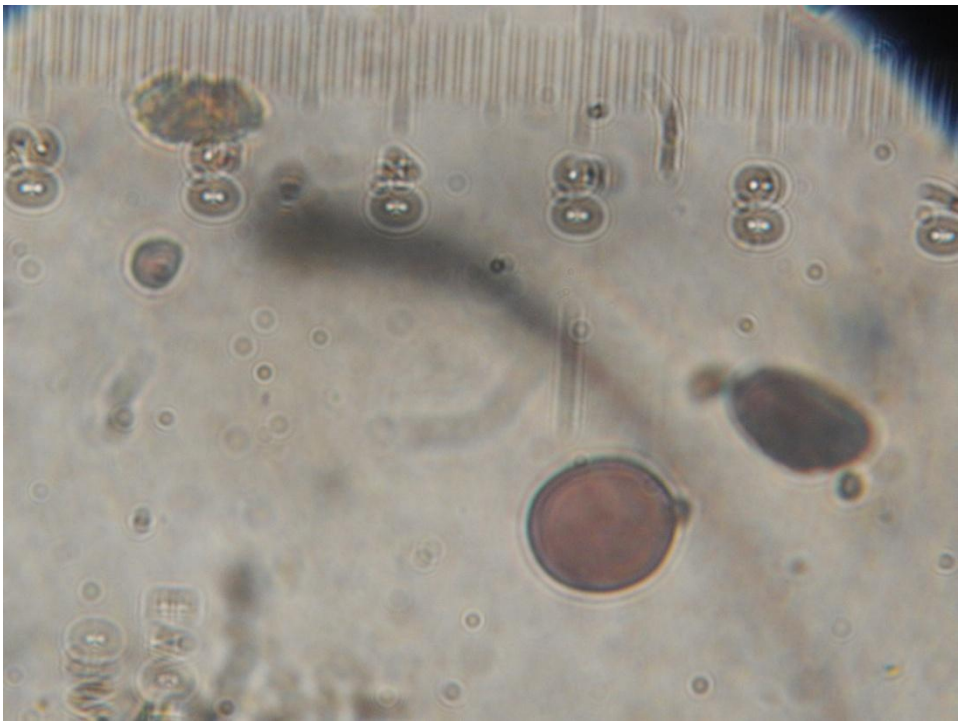

Figure 1(f): A photomicrograph of starch grains (after adding iodine) (100X)

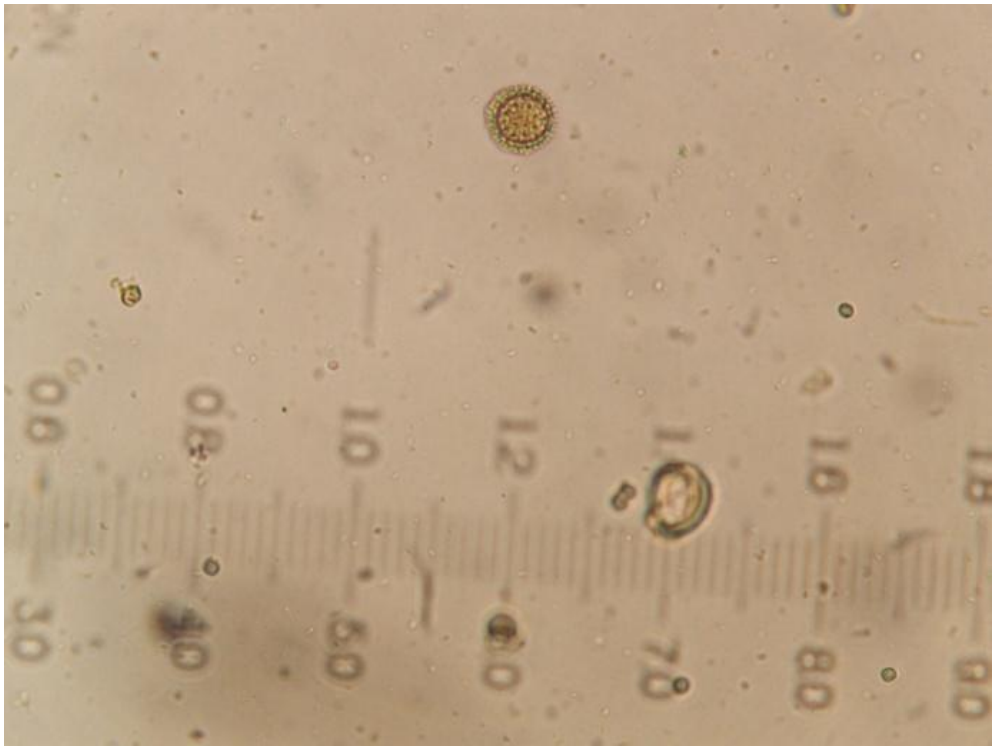

Figure 2(a): A photomicrograph of pollen grains (100X)

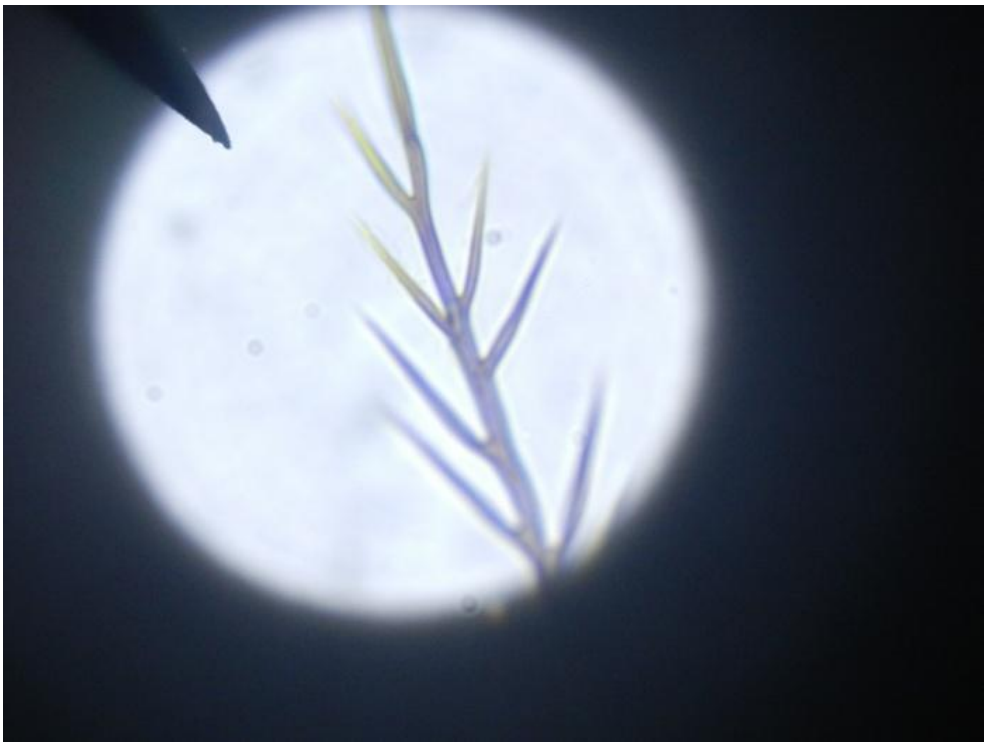

Figure 2(b): A photomicrograph of bee hair (100X)

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