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DRAFT TANZANIA STANDARD

Stinging bee honey – Specification

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Kilombero Sugar Company Limited
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Njiro Bee Research Centre – Arusha
University of Dar es Salaam – Chemistry Department

Tanzania Bureau of Standards
P. O. Box 9524
Dar es Salaam
Tel: 022 2450206/2450298/2450949
Fax: 022 2450959
E-mail: info@tbstz.org

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Stinging bee Honey – Specification

0 Foreword

a natural sweet substance produced by honeybees from the nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of the plants, which honeybees collect, transform and combine with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature

Tanzania has an enormous production potential of liquid bees honey. The two groups of bees that produce harvestable honey in Tanzania are the stinging honeybees (*Apis mellifera*) and the stingless *Trigona* and *Melipona spp*. The former produces honey which is most valued commercially as food; and the latter is mainly valued for its medicinal properties.

The honey produced by stinging bees has grown in importance as a non traditional export product to the international market; as well as to the local consumer. This growing trend has been attributed to the purity and the natural characteristics of the Tanzania honey.

The growing commercial importance of the honey has made it necessary to prescribe this Tanzania Standard in order to ensure safety and quality of the honey produced and traded.

In the preparation of this Tanzania Standard assistance was derived from:

Codex Standard No. 12.2001, *Honey – Specification*, published by the Codex Alimentarius Commission.

Harmonized East African Standard (HEAS) No. 036:1998, *Standard specification for honey*, published by the East African Standards Secretariat.

Sri Lanka Standard 464: 1979, *Specification for bees honey*, published by the Sri Lanka Bureau of Standards.

In reporting the results of a test or analysis made in accordance with this Tanzania Standard, if the final value, observed or calculated is to be rounded off, it shall be done in accordance with TZS 4 (see clause 2).

1 Scope

This Tanzania Standard prescribes the requirements and the methods of sampling and test for honey, produced by stinging honeybees *(Apis mellifera*) intended for direct human consumption and industrial use.

2 Reference

The following referenced documents are indispensable for the application of this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies

TZS 4: , *Rounding off numerical values*

TZS 59: *Water – Distilled quality – Specification*
TZS 76 General method for determination of arsenic silver diethyldithiocarmate photometric method

TZS 109: *Code of hygiene for food establishments*

TZS 538: , *General standard on packaging, marking and labelling of foods*

TZS 119: , *Microbiology – General guidance for enumeration of coliforms – Most probable number technique (MPN)*

TZS 131: , *Method for yeast and mould count – Foodstuffs*

AOAC Official method 999.11 *Determination of Lead, Cadmium, Copper, Iron and Zinc in Foods-Atomic Absorptions Spectrophotometry after dry ashing*

AOAC Official method 985.*16 determination of Tin in canned foods-Atomic Absorption Spectrophotometric Method*
TZS 268 General atomic absorption spectrophotometric method for determination of lead in food stuffs

3 Terms and Definitions

3.1 For the purpose of this Tanzania Standard, the following definitions shall apply:

3.1.1 honey

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

3.1.2 table honey

a freshly harvested bee honey that has not been subjected to any kind of processing or treatment which could alter its natural characteristic;..

3.1.3 industrial honey

honey that may have either been subjected to some kind of processing, treatment or not; but having undergone some chemical change to alter its chemical, physical or organoleptic characteristics

3.1.2 floral honey
is the honey which comes from nectaries of flowers.

3.1.4 honeydew honey
is the honey which comes mainly from secretions of living parts of plants or secretion of plant sucking insects on the living parts of plants.

3.1.5 pressed honey
is honey obtained by pressing broodless combs with or without the application of moderate heat.

3.1.6 extracted honey (centrifugal)
is the honey only obtained by centrifuging decapped broodless combs.

3.1.7 drained honey
is the honey obtained by draining decapped broodless combs.

3.1.8 liquid honey
is honey in liquid or a mixture of liquid and crystallized honey.

3.1.9 comb honey
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;

3.1.10 chunk honey
is honey containing one or more pieces of comb honey;

3.1.11 crystallized or granulated honey
is honey that has undergone a natural process of solidification as a result of glucose crystallization.

3.1.12 creamed (or creamy or set) honey
is honey which has a fine crystalline structure and which may have undergone a physical process to give it that structure and make it easy to spread.

4 Requirements

4.1. General Requirement

4.1.1 Description

Honey is essentially a highly concentrated water solution of different sugars, predominantly glucose and fructose (dextrose and laevulose), with small amounts of other more complex sugars.

4.1.2 Colour

The colour of honey varies from near colourless (water white) to dark brown (amber) depending on source, processing and storage condition it has undergone.

4.1.3Consistency

The consistency can either be fluid, viscous or partly or entirely crystallized due to monohydrate formation from dextrose, dependent mainly on glucose/water ratio.

4.1.4Flavour and aroma

The flavour and aroma vary but are derived from plant origin (floral source); light coloured being mild in flavour compared to dark honey.

Honey shall not have any objectionable flavour, aroma, including smoke or taint obtained from foreign matter during handling, processing and storage. The honey shall not have begun to ferment or effervesce. Honey shall not be heated directly.

4.2 Specific Requirement

Honey shall comply with requirements prescribed in table 1.

Table 1 – chemical requirements

S/N	Characteristic	Requirement		Method of test (see annexes)
		Table honey	Industrial honey	

1	Reducing sugar, as invert sugar, **min**, %	60	65	A.1
2	Sucrose, **max**, %	5	10	A.2
3	Moisture, **max**, %	20	22	A.3
4	Water insoluble solids; a) pressed honey, **max**, % b) others, **max**, %	 0.5 0.1	 0.5 0.1	A.4
5	Mineral content (ash), **max**, %	0.6	0.1	A.5
6	Acidity as: a) milliequivalent acids/kg, **max.**	 40	 50	A.6
7	Diastase activity, **min**	8	10	A.7
8	Fructose/Glucose ratio, **min**	1	1	A.9
9	Fieche's test	Negative	Negative	A.10
10	Hydroxymethyl furfural (HMF), **max**, mg/kg*	40	40	A.11

*Determination shall be performed only when Fiech's test is positive.

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4.2.1 Colour of honey On a Pfund scale the honey colours as determined optically shall be as given in table 2.

Table 2 – Honey colours on Pfund scale

Pfund scale (mm)	Colour designations
0 – 8	Water white
> 8 – 17	Extra white
> 17 – 34	White
> 34 – 50	Extra light amber
> 50 – 85	Light amber
> 85 – 114	Amber
> 114	Dark amber and dark

Note: The colour of table honey shall be uniform throughout and may vary from nearly colourless to dark amber; while industrial honey may be darker in colour and uniform

4.3 Food additives

Honey shall not contain any added substances in any form; including food additives.

4.4 Metallic contaminants

The levels of metallic contaminants in honey shall not exceed those specified in table 3.

Table 3 – Metal contaminants requirement

Metal	Requirement) mg/kg, in max	Methods of test
Lead mg/kg max	1.0	TZS 268
Arsenic mg/kg max	0.5	TZS 76

Copper mg/kg max	2	AOAC 999.11
Tin mg/kg max	5.0	AOAC 985.16
Zinc mg/kg max	5.0	AOAC 999.11

4.5 Pesticides and antibiotics residues

The levels of pesticides and antibiotics residues shall not exceed those prescribed in Codex Almentarius .

5 Hygiene

5.1 The product covered by the provisions of this Tanzania Standard shall be produce in accordance with Good Apiary Practices and as prescribed in TZS 109 (see clause 2).

5.2 Honey shall also be free from mould and coliform bacteria when tested in accordance with TZS 119 and TZS 131 (see clause 2).

5.3 Freedom from foreign matter

Honey shall be free from inorganic and organic matter foreign to its origin from plants, including insects, insect debris, brood, grains of sand or other extraneous matter.

5 Methods of sampling and tests

6 sampling

6.1 In all cases sampling and tests of honey shall be performed as prescribed below and as per the relevant annex A of this Tanzania Standard.

6.1.1 *General requirements for sampling of honey*

In drawing, preparing, storing and handling of samples, the following precautions and directions shall be observed:

a) Samples shall be taken in a protected place not exposed to damp air, dust or soot;

b) The sampling instrument shall be clean and dry when used;

c) Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers for samples, from adventitious contamination;

d) The samples shall be placed in clean and dry glass or food grade plastic containers. The sample containers shall be of such a size that they are almost completely filled by the sample;

e) Each container shall be sealed airtight after filling and marked with all details of sampling, code number and other important particulars of the consignment;

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

6.1.2 *Sampling of liquid, strained or crystallized 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 30 minutes, at 60°C, then if necessary heat at 63°C, until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as sample liquefies. Do not heat honey intended for hydroxymethylfurfural or diastatic determination. Also ensure that as little air as possible is stirred into the honey, especially if the sample is to be used for determination of hydroxymethylfurfural. If foreign matter, such as wax, sticks, bee particles or comb, etc. is present, heat sample at 40°C in water bath and strain through cheese cloth in hot water funnel before sampling or pass through a 0.5 mm sieve. Gently press crystallized honey with a spatula through the sieve.

6.1.3 *Sampling of comb honey*

Cut across top of comb, if sealed, and separate completely from comb by straining through a sieve the mashes of which are made by so weaving wire as to form square opening of 0.500 mm by 0.500 mm. When portions of comb or wax pass through sieve, heat and strain sample as in 6.1.2.

6.1.4 *Scale of sampling*

6.1.4.1 The number of containers to be sampled from each lot shall depend on the size of the lot and it shall be done according to table 5.

6.1.4.2 The containers shall be selected at random from the lot

Lot size (N)	Number of containers to be selected (n)
Up to 25	6
26 – 150	9
151 – 500	12
501 and above	15

6.1.5 *Preparation of composite sample*

Draw with suitable sampling instrument approximately equal quantities of material from different parts of the containers selected as above. These materials shall be mixed together to form a composite sample weighing about 300 g ms. This composite sample shall be divided into two equal parts and transferred to clean and dry containers, sealed air tight and labelled. One of these composite samples is intended for testing and the other for reference.

6.2 Tests

Tests for honey shall be carried out as prescribed in the appropriate annexes given in tables 1 and 3.

7 Packaging, marking and labelling

In addition to the requirements given in TZS 538 (see clause 2), the following specific provisions shall apply:

7.1 Packaging

7.1.1 Honey shall be packed in air tight, hygienically clean, plain, wide mouthed, well sealed glass jars or any other suitable containers which are food grade, acid resistant, non-reactive to the content and cannot cause the transfer of foreign odours to the product. Rephrase in agreed format

7.1.2 Honey for non retail trade shall be packed in steel drums. The steel drums must be food grade quality, coated with safe paint lacquer, clean, moisture-proof and free of residual taste and odour of other products.

7.1.3 The net content shall comply with the Weights and Measures Prepackaging Regulations.

7.2 Marking and labelling – Each container shall be legibly and indelibly marked or labelled with the following particulars:

7.2.1 *Name of the product*

a) The designation name "honey", if it conforms to this Tanzania Standard.

Note

b) No honey may be designated by any of the provision of sections 3.1.1 and 3.1.3, unless it conforms to the appropriate description contained therein. The style given in clause 3.4 shall be declared.

c) Honey may be designated according to the floral or plant source if it comes wholly or mainly from that particular source and has the organoleptic, physico-chemical and microscopic properties corresponding with that origin. No person shall by words, description or otherwise mark or label as honey, a product which is impure or adulterated.

d) Honey complying with provisions of this Tanzania Standard may be sold under designations which describe its physical characteristic, e.g. "creamed", "whipped" or "set".

7.2.2 *Name and address of the prod, packer or exporter*

7.2.3 *Country of origin*

7.2.4 *Code or batch number*

7.2.5 *Date of packing*

7.2.6 *Date of expiry*`

7.3 Outer containers holding prepackaged honey in small units shall be fully labelled or marked.

A

Annex A

Methods of test

A.1 Determination of reducing sugar content

A.1.1 Principle of method

The method is a modification of the Lane and Eynon (1923) procedure involving the 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 of this type of determination is attained by ensuring that the reduction of the Fehling's solution 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.

A.1.2 Reagents

A.1.2.1 *Soxhlet's modification of Fehling's solution*

Solution A: Dissolve 69.28 g copper sulphate pentahydrate ($CuSO_4.5H_2O$; MW = 249.71) with distilled water to 1 litre. Keep one day before titration.

Solution B: Dissolve 346 g sodium potassium tartrate (C_4H_4K; $NaO_6.4H_2O$; MW = 282.23) and 100 g sodium hydroxide (NaOH) with distilled water to 1 litre. Filter through prepared asbestos.

A.1.2.2 *Standard invert sugar solution (10 g/L)*

Weigh accurately 9.5 g pure sucrose, add 5 ml hydrochloric acid (ca. 36.5 percent w/w pure HCl) and dilute with water to about 100 ml, store this acidified solution for several days at room temperature (ca. 7 days at 12°C to 15°C, or 3 days at 20°C to 25°C), and then dilute to 1 litre. (N.B. Acidified 1.0 percent invert sugar remains stable for several months). Neutralize a suitable volume of this solution with 1 M sodium hydroxide solution (40 g/L) immediately before use and dilute to the required concentration (2 g/L) for the standardization.

A.1.2.3 *Methylene blue solution*

Dissolve 2 g in distilled water and dilute to 1 litre.

A.1.2.4 *Alumina cream*

Prepare cold saturated solution of alum ($K_2SO_4Al_2$ $(SO_4)_3.24H_2O$) 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 test for sulphate with barium chloride solution. Pour off excess water and store residual cream in stoppered bottle.

A.1.3 Procedure

A.1.3.1 *Preparation of test sample – First procedure* (applicable to honeys which may contain sediment)

a) Transfer an accurately weighed sample of approximately 25 g (W_1) from the homogenized honey to 100 ml volumetric flask, add 5 ml alumina cream (7.1.2.4); dilute to volume with water at 20°C and filter.

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

Or

A.1.3.2 *Preparation for test sample – Second procedure*

a) Weigh accurately a representative quantity of about 2 g (W_2) of the homogeneous honey sample, dissolve in distilled water and dilute to 200 ml in a calibrated volumetric flask (honey solution).

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

A.1.3.3 *Standardization of the modified Fehling's solution*

Standardize the modified Fehling's solution A so that exactly 5 ml (pipette), when mixed with approximately 5 ml of Fehling's solution B, will react completely with 0.050 g invert sugar added as 25 ml dilute invert sugar solution (2 g/L).

A.1.3.4 Alternatively, pipette 5 ml Fehling's solution A and mix with approximately 5 ml of Fehling's solution B. Then determine the amount (mg) of the invert sugar that will react with the mixture.

Percent reducing sugar = ((D X E)/V/M) x 100

where,

M = mass of honey sample (mg)

D = volume of diluted honey solution which would contain all of the original honey sample on the basis of the solution

V = volume of diluted honey solution used up in the titration to reduce 5 ml of Fehling's solution

E = the amount (mg) of standard invert sugar which reacts with 5 ml of Fehling's solution

A.1.3.4 *Preliminary titration*

The 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 percent 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 into a 250 ml Erlenmeyer flask and add approximately 5 ml Fehling's solution B. Add 7 ml distilled water, a little powdered pumice or other suitable antipumping agent, followed by about 15 ml diluted honey solution from a burette. Heat the cold mixture to boiling over a wire gauze, and maintain moderate ebullition for 2 min. Add 1 ml 0.2 percent 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).

A.1.3.5 *Determination*

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

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

'Ref. ISO 565-1983. Such sieve could be replaced by U.S. sieve with No. 40 standard screen (size of opening 0.420 mm).

Add (25-x) ml distilled water, a little powdered pumice or other suitable antibumping agent and, from a burette, all but 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 2 min. Add 1.0 ml 0.2 percent methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 min, by repeated small additions of diluted honey solution until the indicator is decolorized. Note the total volume of diluted honey solution (y ml). Duplicate titration should agree within 0.1 ml.

A.1.4 Calculation and expression of results

Where the first procedure (A.1.3.1) has been used;

$$C = \frac{25 \times 1000}{W_1 \quad Y_1}$$

Where the second procedure (A.1.3.2) has bee used;

$$C = \frac{2 \times 1000}{W_2 \quad Y_2}$$

where C = g invert sugar per 100 g honey

W_1 = weight (g) of honey sample taken according to sub-section A.1.3.1

W_2 = weight (g) of honey sample taken according to sub-section A.1.3.2

Y_1 = volume (ml) of diluted honey solution consumed in the determination carried out according to the first procedure (A.1.3.1).

Y_2 = volume (ml) of diluted honey solution consumed in the determination carried out according to the second procedure (A.1.3.2).

A.1.5 Notes on the procedure

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. The following table gives typical volumes which may be encountered at the preliminary titration stage for the incremental contents of invert sugar shown, assuming the test sample (A.1.3.1) weighs about 25 g or test sample (7.1.4.2) weighs about 2 g:

Invert sugar content	Volume of distilled water to be added
60	8.3
65	9.6
70	10.7
75	11.6

A.1.6 Standardization of Fehling's solution

Dry a quantity of standard dextrose in a vacuum oven for 2 hours at 100°C and then dissolve exactly 5.0 gram in distilled water, dilute to 500 ml and mix thoroughly. Pipette 25.0 ml of the Fehling's solution into a boiling flask, and then bring to boil and titrate with standard dextrose solution as directed under "procedure". Adjust the concentration of the Fehling's solution by dilution or addition of copper sulphate so that titration requires 12.2 ml of standard dextrose solution.

A.2 Determination of apparent sucrose content

A.2.1 Principle of the method

Based on the Walker (1917) inversion method.

A.2.2 Reagents

A.2.2.1 Soxhlet modification of Fehling's solution (A.1.2.1)

A.2.2.2 Standard invert sugar solution (A.1.2.2)

A.2.2.3 Hydrochloric acid (6.34 M aqueous)

A.2.2.4 Sodium hydroxide solution (5 M aqueous)

A.2.2.5 Methylene blue solution 2 g/l (A.1.2.3)

A.2.3 Procedure

A.2.3.1 *Preparation of test sample*

Prepare the honey sample as in A.1.3.1 (a). Dilute 10 ml of this solution to 250 ml with distilled water: honey solution (for sucrose determination) or prepare the honey solution as in A.1.3.2 (a).

A.2.3.2 *Hydrolysis of the test sample*

The honey solution (50 ml) is placed in a 100 ml graduated flask, together with 25 ml distilled water; heat the test sample to 65°C over a boiling water-flask. The flask is then removed from the water-bath and 10 ml of 6.34 M hydrochloric acid added. The solution is allowed to cool naturally for 15 minutes, and then brought to 20°C and neutralized with 5 M sodium hydroxide, using litmus paper as indicator, cooled again, and the volume adjusted to 100ml (diluted honey solution).

A.2.3.3 *Titration*

As in A.1.3.4 and A.1.3.5.

A.2.4 Calculation and expression of results

Calculate percent invert sugar (g invert sugar per 100 g honey) after inversion using the appropriate formula as for percent invert sugar before inversion in A.1.4.

Apparent sucrose content = (invert sugar content after inversion minus invert sugar content before inversion) X 0.95

The result is expressed as g apparent sucrose/100 g honey.

A.3 Determination of moisture content (Type 1 method)

For routine purposes, oven drying methods may be used for determination of moisture in the honey, but for accurate and reference work, the refractometric method given in this Tanzania Standard shall be used.

A.3.1 Principle of method

Based on the refractometric method of Chataway (1932), revised by Wedmore (1955).

A.3.2 Apparatus

Refractometer

A.3.3 Sampling

The honey is prepared for sampling as in 6.1.1 of this Tanzania Standard.

A.3.4 Procedure

A.3.4.1 *Determination of the refractive index*

Determine the refractive index of the test sample using a refractometer at a constant temperature near 20°C. Convert the reading to moisture content (percent m/m) using the table given below. If the determination is made at a temperature other than 20°C, covert the reading to standard temperature of 20°C, according to the temperature corrections quoted. The method used is to be noted in the test report.

Table 1 – Estimation of moisture content

Refractive index (20°C)	Moisture content (percent)	Refractive index (20°C)	Moisture content (percent)	Refractive index (20°C)	Moisture content (percent)
1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.4
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
1.4946	16.8	1.4845	20.8	1.4740	25.0
1.4940	17.0	1.4840	21.0		
		1.4835	21.2		

A.3.4.2 *Temperature correction – refractive index*

Temperature above 20°C – Add 0.00023 per °C to the refractive index reading per °C
Temperature below 20°C – Subtract 0.00023 per °C to the refractive index reading per °C.

A.4 Gravimetric determination of water-insoluble solids content

A.4.1 Procedure

A.4.1.1 *Preparation of test sample*

Honey (20 g) is weighed to the nearest centigram (10 mg) and dissolved in a suitable quantity of distilled water at 80°C and mixed well.

A.4.1.2 *Gravimetric determination*

The test sample is filtered through a previously dried and weighed fine sintered glass crucible (pore size 15.40) and washed thoroughly with hot water (80°C) until free from sugars (Mohr test). The crucible is dried for one hour at 135°C, cooled and weighed to 0.1 mg.

A.4.1.3 *Expression of results*

The result is expressed as percent water insoluble solids (m/m)

$$= \frac{m_1 - m_2}{m_o} \times 100$$

where,

m_o = mass of honey taken

m_1 = mass of crucible and water insoluble solids

m_2 = mass of crucible

A.5 Determination of mineral content (ash) type 1 method

A.5.1 Sampling

Honey is prepared for sampling as in 6.1.1 of this Tanzania Standard.

A.5.2 Procedure

Ignition of the honey

Honey (5-10 g) is weighed accurately into an ignited and pre-weighed platinum or silica dish and gently heated in a muffle furnace until the sample is black and dry and there is no danger of loss by foaming and overflowing. An infra-red lamp may also be used to char the sample before inserting it into the furnace. If necessary, a few drops of olive oil may be added to prevent frothing. The sample is then ignited 600^{o}C to constant weight. The sample is cooled before weighing.

A.5.3 Expression of results

The result is expressed as percent ash (m/m).

A.6 Determination of acidity

A.6.1 Reagents

A.6.1.1 *Sodium hydroxide 0.1 N (carbonate-free)*

A.6.1.2 *Phenolphthalein indicator 1 percent (m/v) in ethanol, neutralized*

A.6.1.3 *Distilled water made carbon dioxide free by boiling and subsequent cooling*

A.6.2 Procedure

A.6.2.1 *Preparation of test sample*

Honey (10.0g) is weighed accurately and dissolved in 75 ml distilled water (A.6.1.3).

A.6.2.2 *Titration*

The test sample is titrated against carbonate-free 0.1 M sodium hydroxide solution using 4-5 drops of neutralized phenolphthalein indicator. The end-point colour should persist for 10 seconds. For darkly-coloured samples, a smaller weight should be taken. As an alternative, a pH meter may be used and the sample titrated to pH 8.3.

A.6.3 Calculation and expression of results

The result is expressed as millival (milliequivalent) acids/kg honey and is calculated as follows:

Acidity = 10 v

where

v = the number of ml 0.1 M NaOH used in the neutralization of 10 g honey.

Acidity (as formic acid), percent by mass

$$= \frac{0.23 \times V}{m}$$

where

V = volume of ml of 0.1 N NaoH used in the neutralization of the test sample.

m = mass in g of the sample taken from the test.

A.7 Determination of diastase activity

A.7.1 Principle of the method

Based on the method of Schade et al. (1985) modified by White et al. (1959) and Hardon (1961).

A.7.2 Reagents

A.7.2.1 *Iodine stock solution*

Dissolve 8.8 g of iodine analytical grade, in 30-40 ml water containing 22 g potassium iodide, analytical grade, and dilute to 1 litre with water.

A.7.2.2 *Iodine solution 0.0007 N*

Dissolve 20 g potassium iodine, analytical grade, in 30-40 ml water in a 500 ml volumetric flask. Add 5.0 ml iodine stock solution and make up to volume. Make up a fresh solution every second day.

A.7.2.3 *Acetate buffer – pH 5.3 (1.59 M)*

Dissolve 87 g sodium acetate.3H20 in 400 ml water; add about 10.5 ml glacial acetic acid in a little water and make up to 500 ml. Adjust the pH to 5.3 with sodium acetate or acetic acid as necessary, using a pH meter.

A.7.2.4 *Sodium chloride solution 0.5 M*

Dissolve 14.5 g sodium chloride, analytical grade, in boiled-out water and make up to 500 ml. The keeping time is limited by mould growth.

A.7.2.5 *Starch solution*

(a) Preparation of soluble starch

In a conical flask immersed in a water-bath and fitted with a reflux condenser, boil 20 g of potato starch for one hour in the presence of a mixture of 100 ml of 95% ethanol and 7 ml of 1 M hydrochloric acid. Cool, filter through a filtering crucible (pore size 90-150) and wash with water until the wash/water ceases to give any chloride reaction. Drain thoroughly and dry the starch in air at 35°C. The soluble starch must be stored in a well stoppered flask.

(b) Determination of moisture content of soluble starch

Accurately weigh a quantity of approximately 2 g of soluble starch and spread in a thin layer over the bottom of a weighing bottle (diameter 5 cm). Dry for one and a half hours at 130°C. Allow to cool in a desiccator and re-weigh. The weight loss with respect to 100 g represents the moisture content. The moisture content of such starch should be 7-8% m/m depending on the humidity of the air in which the sample has been dried.

(c) Preparation of starch solution

Use a starch with a blue value between 0.5 – 0.55 using a 1 cm cell, as determined by the method below. Weigh out that amount of starch which is equivalent to 2.0 g anhydrous starch. Mix with 90 ml of water in a 250 ml conical flask. Bring rapidly to the boil, swirling the solution as much as possible, heating

over a thick wire gauze preferably with an asbestos centre. Boil gently for 3 min., cover and allow spontaneously to cool to room temperature. Transfer to a 100 ml volumetric flask, place in a water bath at 40°C to attain this temperature and make up to volume at 40°C.

Method for determining blue value of starch

The amount of starch equivalent to 1 g anhydrous starch is dissolved by the above method, cooled and 2.5 ml acetate buffer added before making up to 100 ml in a volumetric flask. To a 100 ml volumetric flask add 75 ml water, 1 ml M hydrochloric acid and 1.5 ml of 0.02 N iodine solution. The add 0.5 ml of the starch solution and make up to volume with water. Allow to stand for one hour in the dark and read in 1 cm cell using a spectrophotometer at 660 nm against a blank containing all the ingredients except the starch solution. Reading on the absorbance scale = Blue value.

A.7.3 Apparatus

A.7.3.1 *Water-bath at 40°C ± 0.2°C*

A.7.3.2 *Spectrophotometer to read at 660 nm*

A.7.4 Procedure

A.7.4.1 *Preparation of test samples*

Honey solution

10.0 g honey is weighed into a 50 ml beaker and 5.0 ml acetate buffer solution is added, together with 20 ml water to dissolve the sample. The sample is completely dissolved by stirring the cold solution. 3.0 ml sodium chloride solution is added to a 50 ml volumetric flask and the dissolved honey sample is transferred to this and the volume adjusted to 50 ml.

> N.B. It is essential that the honey should be buffered before coming into contact with sodium chloride.

Standardization of the starch solution

The starch solution is warmed to 40°C and 5 ml pipetted into 10 ml of water at 40°C and mixed well. 1 ml of this solution is pipetted into 10 ml 0.0007 N iodine solution, diluted with 35 ml of water and mixed well. The colour is read at 660 nm against a water blank using a 1 cm cell.

The absorbance should be 0.760 ± 0.020. If necessary the volume of added water is adjusted to obtain the correct absorbance.

A.7.4.2 *Absorbance determination*

Pipette 10 ml honey solution into 50 ml graduated cylinder and place in 40°C ± 2°C water-bath with flask containing starch solution. After 15 minutes, pipette 5 ml starch solution into the honey solution, mix and start stop-watch. At 5 minutes internals remove 1 ml aliquot and add to 10.00 ml 0.00007 N iodine solution. Mix and dilute to standard volume (see 6.7.5.1). Determine absorbance at 660 nm in spectrophotometer immediately using 1 cm cell. Continue taking 1 ml aliquot at internals until absorbance of less than 0.235 is reached.

A.7.5 Calculation and expression

The absorbance is plotted against time (min) on a rectilinear paper. A straight line is drawn through at least the last three points on the graph to determine the time when the reaction

mixture reaches an absorbance of 0.235. Divide 300 by the time in minutes to obtain the distaste number (DN). This number expresses the distaste activity as ml 1 percent starch solution hydrolyzed by the enzyme in 1 g of honey in 1 h at 40°C. This distaste number corresponds with the Goethe scale number.

Distaste activity = DN = ml starch solution (1 percent/g honey/h at 40°C).

A.8 Determination of relative density

A.8.1 Apparatus

A.8.1.1 *Thermostatically controlled water bath maintainable at + 27°C ± 1°C*

A.8.1.2 *Specific gravity bottle*

A.8.2 Procedure

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°C ± 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.8.3 Calculation

$$\text{Relative density} = \frac{C - A}{B - A}$$

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.

A.9 Determination of fructose-glucose ration

A.9.1 Principle of the method

The glucose portion of the invert sugar content of honey is determined by reacting it with iodine. The fructose content is calculated by subtraction.

A.9.2 Reagents

0.05 N iodine solution
0.01 N sodium hydroxide solution
Standard sodium thiosulphate solution (0.05 N)

A.9.3 Procedure

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

A.9.4 Calculation and expression of results

A.9.4.1 Approximate glucose, percent by mass (g of glucose per 100 g honey)

$$w = \frac{(B - S) \times 0.004\,502 \times 100}{a}$$

where

B = volume of sodium thiosulphate solution required for the blank (ml);

S = volume of sodium thiosulphate solution required for the sample (ml); and

a = mass of honey taken for the test.

A.9.4.2 Approximate fructose, percent by mass (g of fructose per 100g honey)

$$x = \frac{\text{total reducing sugars (c)} - \text{approximate glucose content (w)}}{0.925}$$

A.9.4.3 Actual glucose content (g per 100 g honey), percent (y) = w – 0.012 x, and actual fructose content (g per 100 g honey), percent (z)

$$= \frac{\text{Total reducing sugars} - y}{0.925}$$

A.9.4.4 Fructose-glucose ratio $= \frac{\text{Actual fructose content (z)}}{\text{Actual glucose content (y)}}$

A.10 Fieche's test

A.10.1 Reagent

A.10.1.1 *Resorcinol solution*

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

A.10.2 Procedure

Introduce 10 ml of 50% honey solution into test tube and add 5 ml of ether. Shake gently and allow to stand until ether layer is clear. Transfer 2 ml of this clear ether solution to small test tube and add large drop of recently prepared resorcinol solution. Shake and note colour. Cherry red colour appearing within a minute indicates presence of commercial invert sugar/HMF. Yellow to salmon shades have no significance.

A.11 Aniline chloride test

A.11.1 Apparatus

Spectrophotometer

A.11.2 Reagent

A.11.2.1 *Aniline chloride reagent*

Pipette 50 ml of aniline into a 125 ml Erlenmeyer flask, add 15 ml of hydrochloric acid solution containing 25 percent hydrogen chloride and mix.

Prepare the reagent fresh every week.

Redistill the aniline if its colour is darker than yellow or slight brownish yellow.

Preserve aniline and the aniline chloride reagent in a refrigerator.

CAUTION**:** In using aniline or the reagent, take care to keep from contact with skin. Wash off immediately if contact occurs.

A.11.2.2 *Ethyl acetate butter*

Purify absolute ethyl by refluxing about 100 ml to 1200 ml over 7 g to 8 g of metaphenylenediamine hydrochloride for about 90 min, then distilling, rejecting the first 15 ml of distillate and discontinuing distillation when the volume left in the distillation flask is about 100 ml.

A.11.2.3 *Purified isoamyl alcohol*

Reflux 1 litre of reagent grade isoamyl alcohol over 35 g to 40 g of potassium hydroxide for 60 min to 70 min.

Distill in glass apparatus and reject the first 25 ml of distillate and leave 125 ml of liquid remaining in the distillation flask.

A.11.3 Procedure

On a balance accurate to 0.1 g weigh 50 g of honey into 100 ml of volumetric flask (transfer with water to the flask if necessary). Add 50 ml of water, swirl to dissolve and wash down the neck of the flask. Dilute to the mark and mix again thoroughly. Measure exactly 25 ml of the clear solution into a 100 ml to 125 ml separator funnel. Introduce 25 ml of the purified ethyl acetate into the funnel with a volumetric pipette. Stopper and shake vigorously for 2 min. Allow the layers to separate and draw off the lower aqueous layer any emulsion at the interface. Filter the ethyl acetate layer through a folded filter paper and pipette 10 ml of the clear ethyl acetate extract into a small Erlenmeyer flask (50 ml). Add an equal volume of the purified isoamyl alcohol and swirl to mix.

Introduce into the mixed solution exactly 1 ml of aniline chloride reagent, stopper, mix quickly and note the time. Set the flask aside in the dark at a temperature of 20°C to 25°C for 15 min after the addition of aniline chloride reagent.

Without delay, determine absorbance at 250 ml in the spectrophotometer, the clear solvent mixture (equal volumes of ethyl acetate and isoamyl alcohol) in the standard cell. Multiply absorbance by 100 to obtain the aniline chloride number.

A.12 Determination of hydroxymethylfurfural by spectrophotometric method

A.12.1 Scope

This method prescribes a procedure to determine hydroxymethylfurfural using spectrophotometric method.

A.12.2 Principle

The determination of hydroxymethylfurfural (HMF) content is based on the determination of UV absorbance of HMF at 284 nm. In order to avoid the interference of other components at this wavelength the difference between the absorbances of a clear aqueous honey solution and the same solution after addition of bisulphate is determined. The HMF content is calculated after subtraction of the background absorbance at 336 nm. This method is based on the original work of White.

A.12.3 Definition

The method determines the concentration of 5-(hydroxymethyl-)furan-2-carbaldehyde. The result is usually expressed in milligrammes per kilogramme.

A.12.4 Reagents

A.12.4.1 *Carrez solution I***,** dissolve 15 g of potassium hexacyanoferrate (ll), $K_4Fe(CN)_6.3H_2O$ in water and make up to 100 mL.

A.12.4.2 *Carrez solution II***,** dilute 30 g of zinc acetate, $Zn(CH_3.COO)_2.2H_2O$ and make up to 100 mL.

A.12.4.3 *Sodium bisulphite solution***,** 0.20 g/100 g, dissolve 0.20 g of solid sodium hydrogen sulfite, $NaHSO_3$, (metabisulphite, $Na_2S_2O_5$), in water and dilute to 100 mL. The solution should be prepared on the day of use.

A.12.5 Apparatus

A.12.5.1 *Spectrophotometer*, operating in a wavelength range including 284 nm and 336 nm.

A.12.5.2 *Quartz cell*, 1cm

A.12.5.3 *Vortex mixer*

A.12.5.4 *Filter paper*, general purpose

A.12.5.5 *Beaker*, 50 mL

A.12.5.6 *Volumetric flask*, 50 mL.

A.12.5.7 *Test tube*, 18 mm x 150 mm

A.12.6 Procedure

A.12.6.1 *Sample preparation*

Prepare the sample according to clause 4 of this Tanzania Standard.

A.12.6.2 *Determination*

Accurately weigh approximately 5 g of sample into a 50 mL beaker. Dissolve the sample in approximately 25 mL of water and transfer quantitatively into a 50 mL volumetric flask. Add 0.5 mL of Carrez solution I and mix. Add 0.5 mL of Carrez solution II, mix and make up to the mark with water (a drop of ethanol may be added to suppress foam). Filter through paper, rejecting the first 10 mL of the filtrate. Pipette 5.0 mL in each of two test tubes (18 mm x 150

mm). Add 5.0 mL of water to one of the test tubes and mix well (the sample solution). Add 5.0 mL of sodium bisulphite solution 0.2% to the second test tube and mix well (the reference solution).

Dilution of sample and reference solutions is carried out as follows:

Additions to test tube	**Sample solution**	**Reference solution**
Initial honey solution	5.0 mL	5.0 mL
Water	5.0 mL	-
0.2% sodium bisulphite solution	-	5.0 mL

Determine the absorbance of the sample solution against the reference solution at 284 nm and 336 nm in 1 cm quartz cell within 1 h. If the absorbance at 284 nm exceeds a value of about 0.6, dilute the sample solution with water and reference solution with sodium bisulphite solution to the same extent in order to obtain a sample absorbance low enough for accuracy. If dilution is necessary;

$$\text{the dilution, } D = \frac{\text{Final volume of sample solution}}{10}$$

A.12.7 Calculation and expression of results

The HMF content, expressed in mg/kg, of the sample is calculated using the following formula:

$$\frac{(A_{284} - A_{336}) \times 149.7 \times 5 \times D}{W}$$

where,

A_{284} is the absorbance at 284 nm;

A_{336} is the absorbance at 336 nm;

149.7 is the factor $= \frac{126 \times 1000 \times 1000}{16830 \times 10 \times 5}$;

126 is the molecular weight of HMF;

16830 is the molar absorptivity of HMF at λ = 284 nm;

1000 is the conversion g into mg;

10 is the conversion 5 into 50 mL;

1000 is the conversion g of honey into kg;

5 is the theoretical nominal sample weight;

D is the dilution factor, in case dilution is necessary; and

W is the weight of honey taken.

Results are expressed in mg/kg, to one decimal place.

NOTE – The values with the two methods (HPLC and spectrophotometric) are not significantly different from each other.

