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DRAFT EAST AFRICAN STANDARD

Gum Arabic — Specification

EAST AFRICAN COMMUNITY

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East African Community
P.O. Box 1096,
Arusha
Tanzania
Tel: + 255 27 2162100
Fax: + 255 27 2162190
E-mail: eac@eachq.org
Web: www.eac-quality.net

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Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the Principles and procedures for development of East African Standards.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

The committee responsible for this document is Technical Committee EASC/TC 005, *Food Additives.*

Attention is drawn to the possibility that some of the elements of this document may be subject of patent rights. EAC shall not be held responsible for identifying any or all such patent rights.

Introduction

Gum Arabic is a tree gum exuded by two species of *Acacia Senegal* and *Acacia seyal*. However, the term "Gum Arabic" does not indicate a particular botanical source.

The gum is harvested commercially from wild trees, mostly in Sudan (about 70% of the global supply) and throughout the Sahel, from Senegal to Somalia. The name "Gum Arabic" was used in the Middle East at least as early as the 9th century.

Gum Arabic first found its way to Europe via Arabic ports and retained its name of origin. Gum Arabic is a complex mixture of glycoproteins and polysaccharides, predominantly polymers of arabinose and galactose. It is soluble in water, edible, and used primarily in the food industry as a stabilizer, emulsifier, thickener and encapsulating agent.

Gum Arabic — Specification

1 Scope

This draft East African Standard specifies requirements, sampling and test methods for food grade Gum Arabic obtained from *Acacia senegal* (L.) *Willdenow var*. *senegal or Acacia seyal* (family *Leguminosae*) also known as Acacia gum intended for human consumption.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

AOAC 923.03, *Ash of Flour (Direct Method)*

AOAC 952.13, Arsenic in food — Silver diethyldithiocarbamate method

AOAC 972.25, *Lead in Food: Atomic Absorption Spectrophotometric Method*

FDEAS 1244, *General Standard for the Labelling of Food Additives when sold as such — Requirements*

CAC/GL 50, *General guidelines on sampling*

ISO 4833-1, *Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 degrees C by the pour plate technique*

ISO 4833-2, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 2: Colony count at 30 degrees C by the surface plating technique*

ISO 5984, *Animal feeding stuffs — Determination of crude ash*

ISO 1871, *Food and feed products — General guidelines for the determination of nitrogen by the Kjeldahl method*

DEAS 1327, *Harvesting, handling, processing and storage of Gum Arabic — Code of practice*

ISO 6579–1, *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp*.

ISO/TS 6579–2, *Microbiology of food and animal feed — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 2: Enumeration by a miniaturized most probable number technique*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 21527-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0.95*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
food grade material
material made of substances which are safe and suitable for their intended use and which will not impart any toxic substance or undesirable odour or flavour to the product.

3.2
Gum Arabic (Acacia Gum (A. Senegal))
dried gummy exudates nodules obtained from the stems and branches of *Acacia senegal* (L) Wild*, Acacia seyall* (L) Wild, or of related species of Acacia (Family. *Leguminosae*).

Note 1 to entry: Gum Arabic consists chiefly of a high molecular weight polysaccharides and their calcium, potassium and magnesium salts which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid.

3.3
Exudate
Are a fluid or secretions emitted through pores or a wound for different part of plant and have specific physical and biological characteristics, a process known as exuding or exudation.

3.4
Extraneous matters /foreign matter
all organic and inorganic material other than Gum Arabic

3.5
inorganic matter
stones, glass, pieces of soil and other mineral matter

3.7
organic matter
any animal or plant matter (seed coats, straws, weeds) other than Gum Arabic, inorganic matter and harmful/toxic seeds

4 Requirements

4.1 General requirements

Gum Arabic shall be:

a) either be in form of granular or powder form

b) a pale white to yellowish- orange, brown solid, which breaks with a glassy fracture

c) when ground, the granule is paler and has a glassy appearance odourless and slightly acidic

d) free from extraneous materials such as sand and pieces of bark

e) highly soluble in water, gives up to 50% solution and insoluble in ethanol as in accordance with Annex A

4.2 Specific requirements

Gum Arabic shall comply with the specific requirements given in Table 1 when tested in accordance with the test methods specified therein.

Table 1 — Specific requirements for Gum Arabic

S/N	Characteristic	Requirement	Test method
(i)	Total Gum Arabic (dry basis), % m/m, min.	95	Annex B
(ii)	pH (1 % solution)	4.5 – 7.0	Annex C
(iii)	Loss on drying, (105C, 5hrs).)% m/m, max.	15	Annex D
(iv)	Total ash, % (dry basis), max.	4	Annex E
(v)	Hydrolysis	Pass	Annex H
(vi)	Specific rotation	$[\propto]D_{25c}$ (-22) to (- 34)	Annex I
(vii)	Protein content, %(Nx6.6)	1.58 -2.7 [2 max.]	ISO 1871
(viii)	Acid insoluble ash, percent by mass, Max	0.5	Annex F
(ix)	Insoluble matter, percent by mass, Max	1	Annex G
Residual solvents			
(x)	Methanol, mg/kg, max.	200	Annex J
(xi)	Ethanol, mg/kg, max.	5 000	

4.3 Grades of Gum Arabic

Gum Arabic shall be graded based as in Table 2 below

Table 2 — Grading of Gum Arabic

S/No	Characteristic	Requirements			Test Method
		Grade 1	Grade 2	Grade 3	
(i)	Size (diameter) (mm)	> 4	2 - 4	< 1	Annex N
(ii)	Foreign matter, % m/m, max.	0.5 - 1	1 - 3	3 - 5	Annex K

5 Hygiene

The preparation, handling and storage of Gum Arabic shall be in accordance with EAS 39 and DEAS 1327

5.1 Microbiological requirements

Gum Arabic shall comply with the microbiological requirements given in Table 3 when tested in accordance with the test methods specified therein.

Table 3 — Microbiological limits for Gum Arabic

S/N	Characteristic	Requirement	Test method
(i)	[Total (aerobic) plate count, CFU/g, max.]	10^3	ISO 4833-1 ISO 4833-2
(ii)	*Escherichia coli,* CFU/g	Absent	ISO 7251
(iii)	*Salmonella,* CFU/25 g	Absent	ISO 6579-1
(iv)	Yeasts and moulds, CFU/g, max.	200	ISO 21527-2

6 Contaminants

6.1 Heavy metals

Gum Arabic shall comply with the heavy metal and other contaminants limits given in Table 4 and 5 when tested in accordance with the test methods specified therein.

Table 4 —Heavy metal limits for Gum Arabic

S/N	Characteristic	Maximum limits (mg/kg)	Test method
i)	Arsenic	1	AOAC 952.13
ii)	Lead	2	AOAC 972.25

6.2 Other contaminants

Table 5 — Limits for other contaminants for Gum Arabic

S/N	Characteristic	Requirement	Test method
(i)	Starch and dextrin, mg/kg	Passes test	Annex L
(ii)	Tannin bearing gums, mg/kg	Passes test	Annex M

7 Pesticide residues

Gum Arabic shall comply with the maximum pesticide residue limits as may be established by the Codex Alimentarius Commission data base.

8 Packaging

The product shall be securely packaged in containers made of food grade materials. The packaging shall be able to safeguard the product from contamination and protect its properties throughout the shelf-life period.

8.1 Storage

Gum Arabic should be stored under clean, cool and dry conditions, in sealed packages and in a properly ventilated and well-constructed stores.

9 Weights and measures

The weight of the packages shall comply with the Weights and measures regulations of Partner States.

The weight of the packages shall be done in metric units.

10 Labelling

In addition to the requirements of FDEAS 1244, each packages shall be legibly and indelibly marked with the following:

a) name of the product as “Gum Arabic, Acacia gum’’;

b) species from which the Gum Arabic is obtained

c) nature of the product ‘Food grade’

d) grade of Gum Arabic

e) year of harvest

f) harvest date and processing date

11 Sampling

Gum Arabic shall be sampled in accordance with CXG 50.

Annex A
(normative)

Test for solubility

A.1 Apparatus

A.1.1 Balance.

A.1.2 Test tubes (15 cm X 1.5 cm).

A.1.3 Litmus paper

A.2.Reagents

A.2.1 Distilled water.

A.2.2 Ethanol.

A.3 Procedure

A.3.1: 1g of gum is dissolved in 2 ml of distilled water in test tube. The solution formed, flows readily and is acid to litmus (Litmus paper will turn from blue to red).

A.3.2: 2 ml of absolute 95 % ethanol are added to the gum solution; appearance of a precipitate indicates the insolubility of the gum in ethanol.

Annex B
(normative)

Total Gum Arabic content – HPLC chromatographic profile

B.1 Principle

Determine the percentages of major Gum Arabic (those with analytical standards) using HPLC (Method A). Confirm the presence of each minor Gum Arabic (compounds where analytical standards are not available) using HPLC-MS (Method B). Calculate the concentration of the minor compounds using respective molecular mass corrected UV peak area against the rebaudioside A UV standard curve. Calculate their sum and express the content on the dried basis.

B.2 Method A: Determination of major Gum Arabic by HPLC

B.2.1 Reagents

B.2.1.1 Acetonitrile, HPLC grade with transmittance more than 95 % at 210 nm.

B.2.1.2 Deionized water, HPLC grade

B.2.1.3 Standards (Reference and Quality Control Standards)

NOTE: Standards of other similar products may also be included. The analyst should consider that the inclusion of additional standards will lower the concentration of the mixed standards described in B.2.3.

B.2.2 Equipment

B.2.2.1 HPLC consisting of: a high precision binary pump and an auto sampler (capable of operating at 2 °C – 8 °C)

B.2.2.2 Diode-Array detector @ UV at 210 nm

B.2.2.3 Mass Spectrometric Detector (Electrospray Negative Ionisation over a mass range from 50 to 1500 m/z using a unit mass resolution,

Note: For use in Method B below) connected in series. Agilent 1200 with Waters Quattro or equivalent:

- Column: Luna 5µ C18 (2), 100A, (150 mm x 4.6 mm, 5µm, Phenomenex) or Capcell pak C18 MG II (250 mm x 4.6 mm, 5µm, Shiseido Co. Ltd) or equiv.
- Column temperature: 50 °C
- Auto sampler temperature: 2 °C – 8 °C
- Injection volume: 10 µl
- Solvent A: Deionised or LC-MS grade water (0.2 µm filtered)
- Solvent B: LC-MS grade Acetonitrile (0.2 µm filtered)

B.2.3 Preparation of Gum Arabic standard solutions

B.2.3.1 Prepare individual stock standard solutions (1.5 mg/ml) in water:acetonitrile (7:3)

B.2.3.2 Prepare mixed standard solution (115 µg/ml) by mixing 1.0 ml each individual stock standard solution

B.2.3.3 Prepare Peak Identification Standard Solutions (0.1 mg/ml) from individual stock standard solutions in water:acetonitrile (7:3).

B.2.3.4 Prepare mixed working standard solutions in the range of 20 µg/ml – 100 µg/ml by following appropriate dilution of mixed standard solution (b) with water:acetonitrile (7:3).

B.2.3.5 Prepare quality control and system suitability individual stock standard solutions (1.5 mg/ml) as well as mixed standard solution (115 µg/ml) using standards from a different batch /manufacturer (if available).

B.2.3.6 Prepare quality control mixed working standard solutions (40 µg/ml and 80 µg/ml) and system suitability standard (52 µg/ml) by following appropriate dilutions of mixed standard solution

B.2.4 Preparation of sample solution

Accurately weigh 50 mg (W_{sample}) of sample and quantitatively transfer into a 50-ml volumetric flask. Add about 20 ml of water:acetonitrile (7:3), sonicate and shake well to dissolve the sample and make up to volume.

B.2.5 Procedure

Table 6 — HPLC gradient timetable

Time min	**Solvent A** %	**Solvent B** %	**Flow rate** ml/min
0.00	85.0	15.0	0.3
40.0	70.0	30.0	0.3
60.0	55.0	45.0	0.3
70.0	55.0	45.0	0.3
70.1	85.0	15.0	0.3
80.0	85.0	15.0	0.3

B.2.5.1 Inject peak identification standard solutions, identify peaks and calculate relative retention times (RRT) with respect to rebaudioside A.

B.2.5.2 Inject working mixed standard solutions and construct standard curves for each Gum Arabic. Inject quality control and system suitability standard solutions to ensure a satisfactory working system.

B.2.5.3 Inject prepared samples. Dilute sample solution, if required, to bring the concentration of each analyte within the standard curve range. Make duplicate injections. Deduce concentration of each Gum Arabic from its corresponding standard curve and obtain average concentration in sample solution as C_{sample} (µg/ml).

B.2.5.4 Calculate the concentration of each Gum Arabic in the sample solution using the following formula

$$Concentration\ (\%\ w/w\ ) = Csample\ X\ 100/\mathrm{W\ sample}$$

where,

C_{sample} is the average concentration in micrograms per millilitre of Gum Arabic in the sample solution; and

W_{sample} is the mass of sample in micrograms per millilitre in 1 ml of sample solution (~1 000 µg/ml)

Note: The above calculation will change if additional dilutions were done prior to LC injection. Analyst shall account such dilutions in the calculation.

B.2.5.5 Calculate the percentage of major Gum Arabic in the sample by summation of percentage concentrations of individual Gum Arabic in the sample (A).

NOTE: If the concentration of major Gum Arabic in the sample is less than 95 %, the analyst should perform Method B.

B.3 Method B: Determination of minor Gum Arabic by HPLC-MS

B.3.1 Apparatus

B.3.1.1 HPLC-MS conditions may vary based on the manufacturer and model of the system used. Analyst should set the conditions following the manufacturer's instructions.

B.3.1.2 The mass spectrometer is connected to the HPLC-UV system used in Method A. Analyse the mass spectral data of the minor peaks (major Gum Arabic peaks are identified from RRT in method A). Confirm the presence of each minor Gum Arabic from the observed molecular mass ion and one or more of the following mass spectral diagnostic ions in Table B.1.

After confirming the presence of a minor Gum Arabic, correct its mean peak area (obtained from the UV chromatogram) as described in B.3.2.

B.3.2 Calculation of minor Gum Arabic content

Calculate the molecular mass corrected peak area abundance for each minor Gum Arabic as follows:

$Molecular\ mass\ corrected\ peak\ area = \mathrm{M}x \times \mathrm{MPA} / \mathrm{M}Reb\mathrm{A}$

Where;

Mx is the molecular mass of the minor Gum Arabic;

MRebA is the molecular mass of Rebaudioside A (967 amu); and

MPA is the mean peak area

Deduce the concentration of each minor Gum Arabic in micrograms per millilitre from the UV standard curve of rebaudioside A. Calculate the concentration of each minor Gum Arabic in the sample solution using the following formula:

Minor Gum Arabic concentration (%w/w) = $Conc_{sample} \times 100 / Weight_{sample}$

The above calculation will change if additional dilutions were done prior to LC injection. Analyst shall account for such dilutions in the calculation.

Calculate the percentage of minor Gum Arabic in the sample by summation of percentages of individual minor Gum Arabic in the sample (B).

Determine the total Gum Arabic content using the following formula:

TSG = $(A+B) \times 100 / 100-M$

where:

TSG is the total Gum Arabic content in percent w/w, on the dried basis;

A is the percent major Gum Arabic;

B is the percent minor Gum Arabic; and

M is the percentage loss on drying

Annex C
(normative)

Determination of pH (Potentiometric method)

C.1 Requirements

C.1.1 pH meter

C.1.2 Distilled/deionised water

C.1.3 Absorbent tissue

- **C.1.4** Two standard buffer solutions (high pH and low pH, respectively)

C.2 Procedure

C.2.1 Use a calibrated pH meter and follow the manufacturer's instructions. Each time the electrodes are used, rinse them with distilled or deionised water and carefully blot them dry with clean absorbent tissue. Form a fresh reference electrode liquid junction. Rinse the sample vessel three times with each new solution to be introduced.

C.2.2 Choose two standard buffers to bracket, if possible, the anticipated pH of the unknown. Warm or cool these standards as necessary to match within 2 °C the temperature of the unknown, and initially set the temperature compensator to that temperature. Immerse the electrodes in a portion of the first standard buffer, and following the manufacturer's instructions adjust the appropriate standardization control (knob, switch, or button) until the pH reading is that of the buffer. Repeat this procedure with fresh portions of the first standard buffer until two successive readings are within ± 0.02 pH unit without an adjustment of the standardization control.

C.2.3 Rinse the electrodes, blot dry, and immerse them in a portion of the second standard buffer of lower pH. Do not change the setting of the standardization control. Following the manufacturer's instructions, adjust the slope control (thumbwheel switch, knob, or temperature compensator) until the exact buffer pH is displayed.

C.2.4 Repeat the sequence of standardization with both buffers until the pH readings are within ± 0.02 pH unit for both buffers without any adjustment of either control (the amount of sample to be used in sample preparation is given where applicable in the individual specification).

C.2.5 The pH of the unknown solution may then be measured. The difference between the results of two pH determinations when carried out simultaneously on in rapid succession by the same analyst, under the same conditions, should not exceed 0.05 pH unit.

NOTE: Always re-standardize the instrument after even a short period during which the amplifier is turned off.

Annex D
(normative)

Loss on drying

D.1 Requirements

D.1.1 Weighing bottle, with a stopper or crucible or moisture dish

D.1.2 Air oven

D.1.3 Desiccator

D.1.4 Weighing scale

D.2 Procedure

D.2.1 Sample preparation

Weigh 1 g to 2 g of sample (M1). Tare a glass-stoppered, shallow weighing bottle/crucible/ moisture dish that has been dried for 30 min at 105 ºC. Transfer the sample into the bottle, replace the cover, and weigh the bottle and the sample (M2).

Distribute the sample as evenly as practicable to a depth of about 5 mm, and not over 10 mm.

D.2.2 Drying

Place the bottle with its contents in the drying chamber, removing the stopper and leaving it also in the chamber, and dry the sample at the 105 ºC for 2 h. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in a desiccator. Weigh the cool bottle and its contents (M3).

Calculate the loss on drying from the following equation:

Loss on drying (% w/w) = $M2 - M3 \times 100/M1$

where

M_1 is the mass, in grams, of sample;

M_2 is the mass, in grams, of sample and weighing bottle before drying; and

M_3 is the mass, in grams, of sample and weighing bottle after drying and cooling in a desiccator.

If the sample melts at a temperature lower than 105 ºC, prepare the sample as described above, then place it in a vacuum desiccator containing sulfuric acid. Evacuate the desiccator to 130 Pa (1 mm of mercury), maintain this vacuum for 24 h, and then weigh the dried sample. Calculate the loss on drying using the same equation above.

Annex E
(normative)

Determination of total ash

E.1 Requirement

E.1. 1 Porcelain or silica crucible

E.1. 2 Hot plate/ Bunsen burner

E. 1. 3 Muffle furnace

E. 1. 4 Desiccator

E.2 procedure

E.2.1 Preparation of Crucible: Ignite a porcelain or silica crucible (E.1 .1) in a muffle furnace at 550–600°C for 1 hour to remove impurities, cool it in a desiccator, and weigh it accurately, W1

E.2.2 Sample Preparation: Accurately weigh 2–5 g of the sample into the tared crucible (E.1 .1), W2

E.2.3 Preliminary Charring: Heat the sample on a Bunsen burner or hot plate (E.1 .2) to char the material until it stops smoking.

E.2.4 Incineration (Ashing): Place the crucible in the muffle furnace (E.1 .3) and ignite at 550°C to 600°C for 3 to 6 hours. The sample should appear as a white or grey-white ash, indicating the absence of carbon.

E.2.5 Cooling and Weighing: Transfer the crucible to a desiccator (E.1 .4) to cool to room temperature, then weigh immediately to prevent moisture absorption. W3

E.2.6 Re-ashing: Repeat the ignition (1 hour), cooling, and weighing steps until the difference between two consecutive weighings is less than 1 mg (constant weight).

E. 3 Calculation

The total ash content is calculated as a percentage of the dry matter or original sample:

$$Total\ Ash = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

$$Total\ Ash = \frac{W3-W1}{W2-w1} \times 100$$

Annex F
(normative)

Determination of acid insoluble ash

F.1 Principle

The total ash obtained from a sample is treated with dilute hydrochloric acid (HCl). The acid dissolves soluble minerals (like calcium and magnesium), leaving behind insoluble residue such as sand and silicates. This residue is then filtered, washed, and re-ignited.

F. 2 Reagents and materials

F.2.1 Hydrochloric Acid (HCl): Usually 10% (m/m) or 2N–5N concentration.

F.2.2 Porcelain, platinum, or silica crucible

F.2. 3 Ashless Filter Paper: Typically, Whatman No. 42 or equivalent, which leaves no residue upon ignition.

F. 2. 4 Distilled Water: Hot, for washing out the acid from the residue

F. 2. 5 pH paper

F. 2. 6 Muffle furnace

F. 2. 7 Desiccator

F.3 Procedure

F. 3. 1 Weigh the empty crucible, W_1

F. 3. 2 Accurately weigh 2–5 g of into the tared crucible, W_2

F. 3. 3 Carryout the procedure for total ash as indicated in Annex E

F.3. 4 Acid Treatment: Add 25 mL of dilute HCl (F.2.1) to the crucible (F.2.2) containing the **total ash**. Cover with a watch glass and boil gently on a water bath or hot plate for 5 to 10 minutes.

F. 3. 2 Filtration: Filter the mixture through an ashless filter paper (F.2.3) . Ensure all residue is transferred from the crucible to the paper using hot distilled water.

F. 3. 3 Washing: Wash the residue on the filter paper with hot distilled water until the filtrate is neutral (free from acid). This can be verified using pH paper (F.2.5)

F.3. 4 Drying and Ignition: Place the filter paper containing the residue back into the original crucible. Dry it in an oven (approx. 100°C–110°C), then ignite it in a muffle furnace (F.2.6) at **550°C to 600°C** for 1 to 2 hours until the paper is completely consumed and the residue is carbon-free.

F.3. 5 Cooling and Final Weighing: Cool the crucible in a desiccator (F.2.7) and weigh. Repeat ignition until a constant weight is achieved, W_3

F.4 Calculation Formula

Acid Insoluble Ash$= \frac{Weight\ of\ Acid\ Insoluble\ Ash}{Weight\ of\ sample} \times 100$

Acid Insoluble Ash$= \frac{W3-W1}{W2-W1} \times 100$

Annex G
(normative)

Determination of insoluble matters

G.1 Principle

The method involves hydrating the gum to separate the soluble polysaccharide fraction from the insoluble contaminants, followed by filtration, drying, and gravimetric measurement.

G.2 Requirement

G. 2.1 No. 40 sieve is an ASTM E11 test sieve with a 425µm (inch) nominal opening size

G. 2. 2 Distilled water

G. 2. 3 Sulphuric acid or Hydrochloric acid, 10%

G. 2. 4 Gooch crucible or Ashless filter paper

G. 3 Procedure

G.3.1 Weight empty Gooch crucible (G. 2.4), W_1

G.3.1 **Sample Preparation:** A known quantity of the gum arabic sample Is prepared. If using unground samples, they should be powdered to pass through a No. 40 sieve.

G.3.1 **Weighing:** Accurately weigh 2–5 g of sample into the tared Gooch crucible, W_2

G.3.1 **Dissolution/Acid Treatment:** Dissolved in water, often with the addition of 10% diluted sulfuric acid or hydrochloric acid (G. 2. 3) to aid in breaking down soluble components.

G.3.1 **Filtration:** Filter the mixture through a pre-weighed Gooch crucible (or a similar ash-less filter).

G.3.1 **Washing:** Wash the residue on the filter thoroughly with hot water to remove any remaining soluble gum.

G.3.1 **Drying:** Dry the insoluble residue at 105°C for 3 hours.

G.3.1 **Weighing:** Cool in a desiccator and weigh the residue (W_3).

G.3.1 **Calculation:** Calculate the percentage of insoluble matter based on the initial weight of the sample.

G.4 Calculation

Insoluble Matters$= \frac{Weght\ of\ insoluble\ matters}{Weight\ of\ sample} \times 100$

Insoluble Matters$= \frac{W3 - W1}{W2 - W1} \times 100$

Annex H
(normative)

Determination of hydrolysis in gum Arabic

H1. Requirements

H1.1 Diluted Sulphuric acid, 10%

H.1.2 Barium Carbonate ($BaCO_3$)

H. 1.3 Magnetic stirrer

H.1.4 pH Meter

H.1.5 Rotary evaporator

H.1.6 Methanol

H.1.7 Chromatoplates

H.1.8 Formic acid

H.1.9 Mixture of formic acid, methyl ethyl ketone, tertiary butanol, and water (15:30:40:15 by volume)

H.1.10 mixture of isopropanol, pyridine, acetic acid, and water (40:40:5:20 by volume)

H.1.11 Solution of 1.23 g anisidine in 100ml ethanol

H.1.12 Solution of 1.66 g phthalic acid in 100 ml of ethanol

H.2 Procedure

H.2.1 Boil a mixture of 100 mg of the sample and 20 ml of 10% sulfuric acid (H1.1) for 3h.

H.2.2 Allow to cool and add excess barium carbonate (H1.2), mixing with a magnetic stirrer (H1.3) until the solution is of pH 7, and filter.

H.2.3 Evaporate the filtrate in a rotary evaporator (H1.5) at 30-50° C in a vacuum until a crystalline or syrupy residue is obtained.

H.2.4 Dissolve in 10 ml of 40% methanol (H1.6). This is the hydrolysate.

H.2.5 Place 1 to 10 ml spots of the hydrolysate on the starting line of two chromatoplates (H1.7) and spots containing 1 to 10 mg of arabinose, galactose, rhamnose, and glucuronic acid, expected to be present in the hydrolysate.

H.2.6 Use two solvent systems, one for each plate: A. a mixture of formic acid, methyl ethyl ketone, tertiary butanol, and water (15:30:40:15 by volume), H.1.9, and B. a mixture of isopropanol, pyridine, acetic acid, and water (40:40:5:20 by volume), H.1.10 to develop the plates.

H.2.7 After development, spray with a solution of 1.23 g anisidine (H.1.11) and 1.66 g phthalic acid in 100 ml ethanol and heat the plates at 100 °C for 10 min.

H.2.8 A greenish yellow colour is produced with hexoses, a red colour with pentoses, and a brown colour with uronic acids.

H.2.9 Compare sample spots with those for the solutions of arabinose, galactose, rhamnose, and glucuronic acid. Additional spots corresponding to mannose, xylose, and galacturonic acid should be absent.

Annex I
(normative)

Determination of optical rotation

I.1 Principle

Optical rotation of chemicals is generally expressed in degrees, as either "angular rotation" (observed) or "specific rotation" (calculated with reference to the specific concentration of 1 g of solute in 1 ml of solution, measured under stated conditions). Specific rotation usually is expressed by the term $[\alpha]t_x$, in which t represents, in degrees centigrade, the temperature at which the rotation is determined, and x represents the characteristic spectral line or wavelength of the light used

I.2 Requirement

I.2.1 Polarimeter

I.2.2 Distilled water

I.3 Procedure

I.3.1 Sample Preparation: Prepare a 1% to 10% (w/v) solution of the gum arabic sample in distilled water. A standard concentration used is 1g in 100ml (1%w/w)

I.3.2 Filtration: Due to the potential for turbidity, filter the solution through a 0.8 µm Millipore filter or Whatman No. 42 paper to ensure clarity.

I.3.3 Use a polarimeter, which measures the angle of rotation of plane-polarized light.

I.3.4 Fill a 200 mm (2-decimeter) tube with the solution.

I.3.5 Measure the optical rotation at a standard temperature (e.g., 20°C or 25°C).

I.4 Calculation

The specific rotation $[\alpha]D = \dfrac{Observed\ rotation(\alpha)}{c.l}$

Where C represents concentration in grams per milliliter (g/ml), and l represents length in decimeters (dm).

Annex J
(normative)

Determination of Residual solvents – Methanol and ethanol

J.1.Requirement

J.1.1 Reagent

J.1.1.1 Blank solution, sample with very low solvent content

J.1.1.2 Internal standard solution, 3-methyl-2-pentanone

J.1.1.3 Methanol

J.1.1.4 Distilled water

J.1.2 Method I (Determination carried out in water)

J.1.2.1 Blank solution: Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of distilled water and 1.0 ml of the internal standard solution. Heat at 60 °C for 10 min and shake vigorously for 10 s.

J.1.2.2 Internal standard solution: Add 50 ml of distilled water to a 50-ml injection vial and seal. Accurately weigh and inject 15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

J.1.2.3 Samples: Weigh accurately 0.20-g sample into an injection vial. Add 5.0 ml water and add 1.0 ml of the internal standard solution. Heat at 60 °C for 10 min and shake vigorously for 10 s.

J.1.2.4 Calibration solution: Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of the water and 1.0 ml of the internal standard solution. Weigh the vial accurately to within 0.01 mg. Inject a known volume of the component of interest through the septum and again reweigh the vial. Heat at 60 °C for 10 min and shake vigorously for 10 s.

J.1.3 Method II (Determination carried out in methanol)

J.1.3.1 Internal standard solution: Add 50.0-ml methanol to a 50.0 ml injection vial and seal. Accurately weigh and inject 15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

J.1.3.2 Blank solution: Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of methanol and 1.0 ml of the internal standard solution. Heat at 60 °C for 10 min and shake vigorously for 10 s.

J.1.3.3 Samples: Weigh accurately 0.20-g sample into an injection vial. Add 5.0 ml methanol and add 1.0 ml of the internal standard solution. Heat at 60 °C for 10 min and shake vigorously for 10 s.

J.1.3.4 Calibration solution: Solution A: Add 50.0 ml methanol to a 50.0-ml vial and seal. Accurately weigh, to within 0.01 mg, the vial and inject 50 µl of the component of interest through the septum. Reweigh the vial. Mix well.

J.1.3.5 Weigh into an injection vial, 0.20 g of blank, add 4.9 ml of methanol and 1.0 ml internal standard solution. Introduce 0.1 ml of Solution A into the injection vial. Mix well and heat at 60 °C for 10 min and shake vigorously for 10 s.

J.2 Equipment

Head-space gas chromatograph – FID system

J.3 Procedure

Place the sample, blank and calibration samples in the sample tray of the head-space gas chromatograph – FID system. Analyse using the following analytical conditions.

Column: Fused silica, length 0.8 m, i.d. 0.53mm, coated with DB-wax, film thickness 1 µm

Coupled with: Fused silica, length 30 m, i.d. 0.53 mm, coated with DB-1, film thickness 5 µm

Conditions:

Carrier gas: Helium

- Flow rate: 208 kPa, 5 ml/min

- Detector: FID

- Temperatures

Injector: 140 °C

Oven conditions: 35 °C for 5 min, then 5 °C /min to 90 °C, then 6 min at 90 °C

Detector: 300 °C

- Head space sampler

Sample heating temperature: 60 °C

Sample heating period: 10 min

Syringe temperature: 70 °C

Transfer temperature: 80 °C

Sample gas injection: 1.0 ml in split mode

J.3 Calculation

A x B x C / 50 = mg component per injection vial

where

A is the relative peak area of the component concerned;

B is the mass of internal standard in milligrams; and

C is the calibration factor.

Determination of calibration factors

Method 1:

$C = D \times 50/(E \times (F - G))$

Method 2:

$C = D / (E \times (F - G) \times 10)$

where

D is the mass of sample in milligrams;

E is the mass of internal standard in milligrams;

F is the relative peak area of component for the calibration solution; and

G is the relative peak area of the same component for the blank solution

Annex K
(normative)

Determination of foreign matter

K.1 Requirements

K.1.1 Sampling spears/ sampling shovels

K.1.2 Riffle divider

K.1.3 Weigh balance

K.2 Sampling

K.2.1 Gum Arabic in bags/ containers

K.2.1.1 For lots with not less than 5 bags or containers, the number of bags/ containers to be sampled can be calculated by using the square root rule.

Number of bags to sample = $\sqrt{N}$
Where N = total number of bags in the lot.

Example:
- 100 bags → $\sqrt{100} = 10$ bags sampled
- 400 bags → $\sqrt{400} = 20$ bags sampled

K.2.1.2 Take approximately 200–300 g per selected bag using a sampling spear or sampling shovel.

K.2.1 3 Combine increments into a composite of approximately 2–5 kg.

K.2.1.4 Mix thoroughly and reduce using quartering or a riffle divider.

K.2.1.5 Reduce the sample to 500 g – 1 kg for grading analysis

K.2.2 For Bulk (Loose) Gum in Warehouse

K.2.2.1 Take at least 10–15 incremental samples from different depths and locations (top, middle, bottom) by using sampling spears.
K.2.2.2 Each increment ~200 g

K.2.2.3 Prepare 3–5 kg composite

K.2.2.4 Mix thoroughly and reduce using quartering or a riffle divider to 1 kg for grading analysis.

K.3 Procedure

K.3.1 Spread the 1 kg sample (W_1) evenly on a clean white tray under natural daylight or standardized lighting (not yellow artificial light).

K.3.2 Manually separate visible foreign materials using forceps.

K.3.3 Classify and collect the following foreign matters separately:

K.3.3.1 Bark fragments (A)

K.3.3.2 Stones, Soil particles and sand clumps (B)

K.3.3.3 Insect bodies or fragments (C)

K.3.3.4 Other plant debris (D)

K.3.4 Place all foreign materials into a pre-weighed container.

Foreign matters, % m/m$= \frac{A+B+C+D}{W1} \times 100$

Annex L
(normative)

Determination of Starch and dextrin

L.1 Apparatus and equipment

L.1.2 Analytical balance.

L.1.3 Conical flasks.

L.1.4 Hot plate with magnetic stirrer.

L.2 Chemicals and reagents

L.2.1 Distilled water.

L.2.2 Iodine Test Solution

Iodine (1.4 grams) is dissolved in a solution of 3.6 grams of potassium iodide in 10 mls distilled water, hydrochloric acid (0.3 grams) is added and made to 100 mls with distilled water.

L.3. Procedure

L.3.1 2% of gum solution is boiled, allowed to cool then few drops of iodine test solution (H.2.2) are added.

L.4. Interpretation of Results

No bluish or reddish color should be produced

Annex M
(normative)

Determination of tannin

M.1 Apparatus and equipment

M.1.1 Analytical balance.

M.1.2 Conical flask.

M.2 Chemicals and reagents

M.2.1 Distilled water.

M.2.2 Ferric Chloride Test Solution

An aqueous solution (6% w/v) of Ferric Chloride Hexahydrate (FeCl3.6H2O)

M.3 Procedure

M.3.1 To 10 mls of 2% solution of the gum sample, add about 0.1 ml of FeCl3 solution (I.2.2) is added.

M.4. Interpretation of Results

No blackish colouration or blackish precipitate should be formed indicating absence of tannins.

Annex N
(normative)

Determination of Gum Arabic sizes for grading

N.1 Requirements

N.1.1 Certified stainless steel sieves: 4.0 mm, 2.0 mm, 1.0 mm (round hole seives)

N.1.2 Bottom pan

N.1.3 Mechanical sieve shaker (preferred)

N.1.5 Analytical balance (±0.01 g)

N.1.6 Clean receiving trays

N. 2 Procedure

N.2.1 Stack sieves in descending order:

a) Top: 4.0 mm

b) Next: 2.0 mm

c) Next: 1.0 mm

d) Bottom: pan

N.2.2 Place a 1,000 g sample on 4 mm sieve.

N.2.3 Operate the mechanical shaker or shake manually for 5–10 minutes.

N.2.4 Avoid excessive vibration that may fracture tears.

N.2.5 Carefully remove each sieve and collect fractions.

N.3 Fraction identification

a) W1 = retained on 4 mm sieve (>4 mm)

b) W2 = retained on 2 mm sieve (2–4 mm fraction)

c) W3 = retained on 1 mm sieve (1–2 mm fraction)

d) W4 = material in pan (<1 mm fraction)

N.4 Calculation

Percentage of each fraction:

$\% \text{ Fraction} = (W_i / 1000) \times 100$

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