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DRAFT EAST AFRICAN STANDARD

Food grade aspartame — Specification

EAST AFRICAN COMMUNITY

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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

Aspartame (3-Amino-N-(α -carbomethoxy-phenethyl)-succinamic acid, N-L- α -aspartyl-L-phenylalanine-1-methyl ester, $C_{14}H_{18}N_2O_5$) is a low calorie artificial sweetener, sugar substitute and flavour enhancer. It is 100 – 200 times sweeter than sucrose. It is one of the most popular artificial sweeteners and it is widely used in the preparation of beverages, desserts, sweets, dairy products, chewing gums, energy-reduced and weight control products, as a table-top sweetener and in the preparation of food for diabetics.

Aspartame is made of two naturally occurring amino acids: phenylalanine and aspartic acid. The phenylalanine is slightly modified by adding a methyl group to give the product its sweet taste. In the gastrointestinal tract, aspartame is rapidly and completely hydrolysed to phenylalanine, aspartic acid and methanol. The products of hydrolysis are absorbed and enter normal endogenous metabolic pathways.

In the Codex Alimentarius Commission International Numbering System, aspartame is assigned as INS 951 and the FAO/WHO Joint Experts Committee on Food Additives (JECFA) established the Acceptable Daily Intake (ADI) for aspartame at 0 mg/kg – 40 mg/kg body weight.

The use of aspartame as a sugar replacer remains a controversial topic with some scientific studies validating its safety while others suggest it could have effects on the consumer. This standard has therefore been developed to ensure that the aspartame traded and/or used in food products in the East African Community is safe for human consumption.

Food grade aspartame — Specification

1 Scope

This Draft East African Standard specifies requirements, sampling and test methods for food grade aspartame for the food industry.

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 999.11, *Determination of Lead, Cadmium, Copper, Iron, and Zinc in Foods, Atomic Absorption Spectrophotometry after Dry Ashing*

CODEX STAN 107, *General standard for the labelling of food additives when sold as such*

CAC/GL 50, *General guidelines on sampling*

AOAC 952.13, *Arsenic in food. Silver diethyldithiocarbonate method*

EAS 39, *Hygiene in the food and drink manufacturing industry — Code of practice*

CODEX STAN 193, *General standard for contaminants and toxins in food and feed*

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>

3.1

food grade material

material, made of substances that 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

aspartame

low calorie, artificial sweetener and flavour enhancer derived from phenylalanine and aspartic acid

3.3**artificial sweetener**

synthetic substance used as a sugar substitute to sweeten food and drink

4 Requirements**4.1 General requirements**

Food grade aspartame shall be:

- a white, odourless, crystalline powder with a strong sweet taste; and
- Slightly soluble in water and practically insoluble or insoluble in ethanol.

A saturated aqueous solution of the product shall be acidic.

4.2 Specific requirements

Food grade aspartame 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 food grade aspartame

S/N	Characteristic	Requirement	Test method
i)	Purity as $C_{14}H_{18}N_2O_5$, % m/m (dry basis)	98 – 102	Annex A
ii)	Loss on drying, %m/m, max.	4.5	Annex B
iii)	Sulphated ash, % m/m (dry basis), max.	0.2	Annex C
iv)	pH (0.8 % solution)	4.5 – 6.0	Annex D
v)	5-Benzyl-3,6-dioxo-2-piperazineacetic acid, (diketopiperazine), % m/m, max.	1.5	Annex E
vi)	Test for amine group	To pass test	Annex F
vii)	Test for ester group	To pass test	Annex G

5 Hygiene

Food grade aspartame shall be manufactured and handled in accordance with EAS 39.

6 Contaminants

Food grade aspartame shall comply with the Maximum Levels of contaminants given in Table 2 when tested in accordance with the test methods specified therein.

Table 2 — Maximum limits for metal contaminants in food grade aspartame

S/N	Contaminant	Limit	Test method
i)	Lead (as Pb), mg/kg, max.	1	AOAC 999.11
ii)	Arsenic (as As), mg/kg, max.	3	AOAC 952.13

7 Packaging

The product shall be securely packaged in containers made of food grade materials. The packages shall preserve the quality and safety of the product and preclude contamination from the external environment.

8 Weights and measures

The products shall comply with the Weights and Measures Regulations of respective Partner States.

9 Labelling

In addition to the requirements of CODEX STAN 107, the product packages shall be legibly and indelibly labelled with the following information:

- name of the product as “Food grade aspartame”;
- name and physical address of the processor/packer/importer;
- date of manufacture;
- expiry date; and
- net weight of the product in metric units.

10 Sampling

Representative samples of the product shall be drawn in accordance with CAC/GL 50.

Annex A (normative)

Assay: Test for purity

A.1 Apparatus

A.1.1 Titration vessel

A.1.2 Aluminium foil

A.2 Reagents

A.2.1 Dimethylformamide

A.2.2 Thymol Blue

A.2.3 Lithium Methoxide/Sodium Methoxide

A.3 Procedure

Accurately weigh 150 mg of the sample, previously dried at 105 °C for 4 hours. Dissolve in 35 ml of dimethylformamide, add 5 drops of thymol blue, and titrate with a micro burette to a dark blue end-point with 0.1N lithium methoxide or sodium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1N lithium methoxide/sodium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

Caution: Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminium foil while dissolving the sample and during the titration.

Annex B (normative)

Loss on drying

B.1 Apparatus

B.1.1 Titration vessel

B.1.2 Aluminium foil

B.2 Reagents

B.2.1 Dimethylformamide

B.2.2 Thymol Blue

B.2.3 Lithium Methoxide/Sodium Methoxide

B.3 Procedure

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 °C for 2 hours. 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 (M_3).

Calculate the loss on drying from the following equation:

$$\text{Loss on drying (\%w/w)} = \frac{M_1 - M_2}{M_1} \times 100$$

where

M_1 is the mass of sample in grams;

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

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

If the sample melts at a temperature lower than 105 °C, 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 24h, and then weigh the dried sample. Calculate the loss on drying using the same equation above.

Annex C (normative)

Determination of sulphated ash

C.1 Apparatus

C.1.1 Platinum dish

C.1.2 Desiccator

C.2 Reagents

C.1.1 Dilute Sulphuric acid – 10 percent (m/v)

C.2 Procedure

Transfer about 2 g of the sample, accurately weighed, to a tared 50-ml to 100-ml platinum dish or other suitable container and add sufficient dilute sulphuric acid to moisten the entire sample. Heat gently, until the sample is dry and thoroughly charred, then continue heating until all the sample has been volatilized or nearly all of the carbon has been oxidized. Cool, moisten the residue with 0.1 ml of sulphuric acid, and heat in the same manner until the remainder of the sample and any excess sulphuric acid have been volatilized. Finally ignite in a muffle furnace at $800\text{ }^{\circ}\text{C} \pm 25\text{ }^{\circ}\text{C}$ for 15 minutes. Cool in a desiccator and weigh.

C.3 Calculation

$$\text{Sulphated ash, percent by mass} = \frac{M_1}{M} \times 100$$

where

M_1 is the mass, in grams, of residue after igniting, and

M is the mass, in grams, of the sample tested.

Annex D (normative)

Determination of pH (Potentiometric method)

D.1 Apparatus

- D.1.1 pH meter
- D.1.2 Distilled/deionised water
- D.1.3 Absorbent tissue
- D.1.4 Two standard buffer solutions (High pH and low pH, respectively)
- D.1.5 Weighing scale
- D.1.6 Erlenmeyer flasks

D.2 Sample preparation

Prepare a 1:125 solution.

Measure 1 g of sample into a 250-ml Erlenmeyer flask. Add 50 ml of distilled water and dissolve the sample. Add distilled water to the mixture till the 125ml mark and agitate the flask to obtain a uniform mixture.

D.3 Procedure

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.

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.

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.

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).

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 E (normative)

Test for 5-Benzyl-3,6-dioxo-2-piperazineacetic acid

E.1 Apparatus

E.1.1 Gas Chromatograph - of a suitable type, equipped with a hydrogen flame ionization detector and designed for handling glass columns with on-column injection (Micro-Tek 220 or equivalent), containing a 1.83 m x 4 mm (inside diameter) glass column packed with 3 percent OV-1 on 80/100-mesh supelcoport. Condition the column overnight at 250 °C before readjustment and equilibration to the operating condition. To preclude build-up of silicon oxide, clean the detector with acetone frequently.

E.1.1.1 Operating conditions - the operating parameters may vary depending on the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

- a) Column temperature 200 °C
- b) Inlet temperature 200 °C
- c) Detector temperature 275 °C
- d) Carrier gas – Nitrogen, flowing at a rate of 75 ml per minute
- e) Hydrogen and air flow to burner – optimised to give maximum sensitivity
- f) Recorder – 1 mV full scale

E.2 Reagents

E.2.1 Sililation reagent: Just before use, dilute 3 parts, by volume, of N, O-bis-(trimethylsilyl) acetamide with 2 parts of dimethylformamide.

E.2.2 Standard preparation: Transfer about 25 mg of 5-Benzyl-3, 6-dioxo-2-piperazineacetic acid Reference Standard, accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Pipet 10 ml of this solution into a second 100-ml volumetric flask, dilute to volume with methanol, and mix. Pipet 3 ml of the second solution into a 2-dram vial, with Teflon-lined cap, and evaporate to dryness on a steam bath. Add 1 ml of the Silylation reagent to the residue, cap the vial tightly, shake and heat in an oven at 80 °C for 30 minutes. Remove the vial from the oven, shake for 15 seconds, and cool to room temperature.

E.2.3 Sample preparation: Transfer about 10 mg of the sample, accurately weighed, into a 2-dram vial, with Teflon-lined cap, add 1 ml of the Silylation reagent, cap tightly, shake, and heat in an oven at 80 °C for 30 minutes. Remove the vial from the oven, shake for 15 seconds, and cool to room temperature.

E.3 Procedure

Inject a 3 µl portion of the standard preparation into the gas chromatograph and obtain the chromatogram. Measure the height of the peak produced by the 5-benzyl-3, 6-dioxo-2-piperazineacetic acid, and record it as *P*. Under the stated conditions, the elution time is about 7-9 min. Similarly, inject a 3 µl portion of the sample preparation, obtain the chromatogram and measure the height of the peak produced by any 5-benzyl-3,6-dioxo-2-piperazineacetic acid contained in the sample, and record it as *p*.

The percentage of 5-Benzyl-3, 6-dioxo-2-piperazineacetic acid in the sample can be computed as

$$\frac{3 \times M \times p}{500 \times m \times P}$$

where,

M is the mass, in milligrams, of the reference standard taken

m is the mass in milligrams, of aspartame analysed

p is the height of peak produced by 5-benzyl-3, 6-dioxo-2-piperazineacetic acid contained in sample;
and

P height of peak produced by 5-benzyl-3, 6-dioxo-2-piperazineacetic acid contained in standard.

Annex F (normative)

Test for amine group

F.1 Apparatus

F.1.1 Test tube

F.2 Reagents

F.2.1 Ninhydrin

F.2.2 Dimethylsulfoxide

F.2.3 Hydrindantin

F.2.4 4M Lithium acetate

F.2 Procedure

Dissolve 2 g of ninhydrin in 75 ml of dimethylsulfoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4M lithium acetate buffer solution (pH 9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.

Annex G

(normative)

Test for ester group

G.1 Reagents

- G.1.1** Methanol
- G.1.2** Methanol saturated with hydroxylamine hydrochloride
- G.1.3** 5N potassium hydroxide
- G.1.4** Hydrochloric acid
- G.1.5** Ferric chloride

G.2 Procedure

Dissolve about 20 mg of sample in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, and then add 0.3 ml of 5N potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid, and add 0.1 ml of ferric chloride. A burgundy/maroon colour is produced.

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