DRAFT TANZANIA STANDARD

Aspartame (food grade) — Specification

TANZANIA BUREAU OF STANDARDS

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0. Foreword

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 in aspartame is slightly modified by adding a methyl group giving the product its sweet taste. Just like proteins, aspartame is digested once it reaches the intestines. It is fully broken down to aspartic acid and phenylalanine, which are absorbed into the body. In addition, the methyl group from the modified phenylalanine is released in the gut to form methanol. The methanol is absorbed and most of it used to produce energy.

In preparation of this draft Tanzania standard assistance was drawn from India standard;
IS13657 Food grade Aspartame – Specification published by Bureau of India Standards (BIS)

This standard has been developed to ensure that the usage of aspartame in food products conforms to acceptable limits.

In reporting the result of a test or analysis made in accordance with the 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 draft Tanzania Standard specifies requirements, sampling and test methods for food grade aspartame.

2 Normative references

The following referenced documents are referred to in the text in such a way that some or all 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*

TZS 539, Methods of test for the assessment of odors and taint from packaging materials used for food stuffs

TZS 538, Labelling of pre\-packaged foods — General requirements

TZS 116, *General standard for the labelling of food additives when sold as such*

TZS 109, Food processing units – Code of hygiene

CAC/GL 50, *General guidelines on sampling*

ISO 760, *Determination of water — Karl Fischer Method (General method)*

TZS 4, Rounding off numerical values

3 Terms and definitions

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

3.1 aspartame is an odorless low calorie artificial sweetener, sugar substitute and flavor enhancer

3.2 artificial sweetener is artificial substance that has a similar taste to sugar other than sugar used to sweeten food or drink.

3.3 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

4 Requirements

4.1 General requirements

4.1.1 Aspartame shall be, free from off odors, foreign matter and adulterants.

4.1.2 Food grade aspartame shall be:

a) a white, odorless, crystalline powder with a strong sweet aroma; and

b) slightly soluble in water and ethanol/methanol;

4.1.3 A saturated aqueous solution of the Food grade aspartame 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 C14H18N2O5, % m/m (dry basis)	98.0 – 102.0	Annex A
ii)	Moisture %m/m, max.	4.3	ISO 760
iii)	Sulphated ash, % m/m (dry basis), max.	0.2	Annex B
iv)	pH (0.8% solution)	4.5 – 6.0	Annex C
v)	5-Benzyl–3,6–dioxo–2–piperazineacetic acid, (diketopiperazine), %m/m, max.	1.5	Annex D
vi)	Test for amine group	To pass test	Annex E
Vii)	Test for ester group	To pass test	Annex F

5. Food additives

Only the food additives permitted by CODEX STAN 192 in the manufacture of food grade aspartame may be used without exceeding the limits.

6. Hygiene

6.1 Food grade aspartame shall be prepared and packaged in premises built and maintained under hygienic condition in accordance with TZS 109.

6.2 Food grade aspartame shall be free from pathogenic organisms.

6.3 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 contaminants in food grade aspartame

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

7. Sampling

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

8. Packaging, marking and labelling.

8.1 The product shall be securely packaged in containers made of food grade materials conforming to TZS 539. The packages shall preserve the quality of the product, prevent entry of light and preclude contamination from the external environment.

8.2 Marking and labelling

8.2.1 In addition to the requirements of TZS 538 and TZS 116, the product label shall be legibly and indelibly labelled with the following:

(i) Name of the product as Aspartame with the words ‘Food Grade’;
(ii) Name and physical address of the manufacturer/distributor;
(iii) Net weight in metric units;
(iv) Batch/lot number;
(v) Directions for storage;
(vi) Date of manufacture; and the
(vii) Expiry date.
(viii) Instruction for use (refer codex stan for food additives when sold as such)
(ix) Statutory warnings (For industrial use)

8.2.2 The language on the label shall be “Kiswahili” or Kiswahili and English. A second language may be used depending on the designated market.

8.2.3 The packages of the buns may also be marked with the TBS certification mark.

NOTE - The TBS Standards Mark of Quality may be used by the manufacturers only under license from TBS. Particulars of conditions under which the licences are granted, may be obtained from TBS

Annex A

(normative)

Assay: Test for purity

A.1 Reagents

A.1.1 Dimethylformamide

A.1.2 Thymol Blue

A.1.3 Lithium Methoxide/Sodium Methoxide

A.2 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 C14H18N2O5.

Annex B

(normative)

Determination of sulphated ash

B.1 Reagents

B.1.1 Dilute Sulphric acid – 10 percent (m/v).

B.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 °C ± 25 °C for 15 minutes. Cool in a desiccator and weigh.

B.3 Calculation

Sulphated ash, percent by mass=

$$\frac{M1 \times 100}{M}$$

where

*M*1 is the mass, in grams, of residue after igniting, and

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

Annex C

(normative)

Determination of pH

C.1 Procedure

Dissolve 1 g of sample in 125 ml of distilled water and measure the measure the pH of the solution using a calibrated pH meter.

Annex D

(normative)

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

D.1 Apparatus

D.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.

D.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

- Carrier gas – Nitrogen, flowing at a rate of 75 ml per minute

- Hydrogen and air flow to burner – optimised to give maximum sensitivity

- Recorder – 1 mV full scale

D.2 Reagents

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

D.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.

D.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.

D.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*.

D.4 Calculation

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 E

(normative)

Test for amine group

E.1 Procedure

Dissolve 2 g of ninhydrin in 75 ml of dimethyl sulfoxide, 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 F

(normative)

Test for ester group

F.1 Reagents

F.1.1 Methanol

F.1.2 Methanol saturated with hydroxylamine hydrochloride

F.1.3 5N potassium hydroxide

F.1.4 Hydrochloric acid

F.1.5 Ferric chloride

F.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.

Bibliography

[1] IS 13657: 1993 (Reaffirmed in 2003), Aspartame, food grade — Specification

[2] JECFA monograph, Aspartame

[3] Food chemicals codex (5th Edition)

[4] EFSA Journal (2013) – Scientific opinion on aspartame