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DRAFT TANZANIA STANDARD

Fermented (non-alcoholic) cereal beverages — Specification

TANZANIA BUREAU OF STANDARDS

Fermented (non-alcoholic) cereal beverages — Specification

0. Foreword

A non-alcoholic traditional beverage made by fermented cereals like sorghum, maize and millet resulting in a variety of tastes and textures and are consumed worldwide.

This Tanzania Standard was developed in order to safeguard the consumer and ensure that fermented (non-alcoholic) cereal beverages are safe and of good quality.

In the preparation of this Tanzania Standard, considerable assistance was derived from:
UG 872:2020, *Fermented (non-alcoholic) cereal beverages — Specification*, published by the Uganda National 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.

1 Scope

This Tanzania Standard specifies requirements, sampling and test methods for fermented (non- alcoholic) cereal beverages.

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.

CAC/GL 50, General guidelines on sampling

TZS 2426-1/ISO 21527-1, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1, Colony count technique in products with water activity greater than 0.95

CXS 192, *General standard for food additives*

TZS 109, *General principles of food hygiene -Code of practice*

TZS 122-1:2018/ISO 6579-1: 2017 - *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella- Part 1: Detection of Salmonella spp*.

TZS 268, *General atomic absorption spectrophotometric method for determination of lead in food and food stuffs*

TZS 471 /EAS 104, *Alcoholic beverages — Methods of sampling and test*

TZS 481, *Nutrition labelling — Requirements*

TZS 538, *Labelling for pre-packaged foods — General requirements*

TZS 550, *Use of nutrition and health claims — Requirements*

TZS 731/ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

TZS 789, *Potable water — Specification*

TZS 799/ ISO 16050, *Foodstuffs — Determination of aflatoxin B1, and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products — High-performance liquid chromatographic method*

TZS 1488/ ISO 750, *Fruits, vegetables and derived products – Sampling and methods of test – Determination of titratable acidity*

TZS 1501/ ISO 6637, *Fruits, vegetables and derived products – Sampling and methods of test – Determination of mercury content – Flameless atomic absorption method*

TZS 1502/ ISO 6634, *Fruits, vegetables and derived products – Sampling and methods of test - Determination of arsenic content - Silver diethyldithocarbamate spectrophotometric method*

TZS 118-2, *TZS 118-2: 2018/ISO 4833-2: 2013- Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 2 Colony count at 30 °C by the surface plating technique*

TZS 730- 2/ ISO 16649 (Part 2), *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of -b-glucuronidase-positive Escherichia coli – Part 2 – Colony-count technique at 44 0C using 5-bromo-4-chloro-3-indolyl-b-D-glucuronide*

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 cereal

grains of grasses such as wheat, millet, sorghum, oats, or corn, the starchy grains of which are used as food.

3.2 fermented (non-alcoholic) cereal beverage

acidic non-alcoholic beverage produced by lactic acid fermentation of milled cereal and/or milled cereal (the fermentation being carried out without the addition of any acid other than lactic acid and with the addition of wheaten products)) and that consists wholly or partially of gelatinized milled cereal acidified by the acids introduced or produced during the lactic acid fermentation process, and that may contain permitted sweeteners, edible protein(s), minerals or vitamins (or a combination of these)

3.3 lactic acid fermentation technology

fermentation process involving the activities of a group of gram-positive, non-sporing, non-motile, catalase-negative, bacteria, which ferment carbohydrates to produce lactic acid as the sole or major organic acid

4 Requirements

4.1. Ingredients

a) Cereal or other raw material used in the production of the fermented (non-alcoholic) cereal beverages shall be clean and sound when analysed using sensory attributes and fit for use in the preparation of products for human consumption. Ingredients used shall conform to the corresponding national standard.

b) The following ingredients shall be used in the preparation of fermented (non-alcoholic) cereal beverages:

   i. cereal flour complying with relevant standards;

   ii. sugar;

   iii. yeast;

   iv. lactic acid culture; and

   v. potable water complying with TZS 789

4.2 General requirements

4.2.1 Fermented (non-alcoholic) cereal beverages shall be practically free from off-odours and off-flavours when judged using normal sensory tests.

4.2.2 Fermented (non-alcoholic) cereal beverages shall be practically free from filth (impurities of animal origin, including dead insects) when judged using the normal senses.

4.2.3 Fermented (non-alcoholic) cereal beverages shall be practically free from objectionable matter.

4.2.4 The appearance and consistency of fermented (non-alcoholic) cereal beverages shall be uniform and characteristic of the product.

4.3 Specific requirements

Fermented (non-alcoholic) cereal beverages 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 fermented (non-alcoholic) cereal beverages

S/N	rameter	quirement	Method of Test
	tal solids content, %, (m/m), min	8	nex A
	dity, (%, m/m, as lactic acid), max.	4	TZS 1488
	ohol content, %, (m/m), max.	0.5	TZS 471

4.4 Nutrients

Nutrients including vitamins, minerals and specific amino acids may be added to fermented non-alcoholic cereal beverages in conformity with the requirements stipulated in National legislation.

5. Food additives

5.1 Fermented (non-alcoholic) cereal beverages may contain only permitted additives in accordance with CXS 192.

5.2 Use of non-nutritive sweeteners is not permitted in this product.

6. Contaminants

6.1 Heavy metals

Fermented (non-alcoholic) cereal beverages shall not contain heavy metal contaminants in excess of the limits stipulated in Table 2.

Table 2 – Maximum limits for heavy metal contaminants in fermented (non-alcoholic) cereal beverage

Contaminant	Maximum limit	Method of test
Arsenic (As), mg/kg	0.05	TZS 1502
Lead (Pb), mg/kg	0.05	TZS 268

Mercury (Hg), mg/kg	0.001	TZS 1501
Cadmium (Cd), mg/kg	0.003	TZS 1581-2

6.2 Pesticide residues

The product shall comply with those maximum residue limits established by the Codex Alimentarius Commission for this product.

6.3 Mycotoxins

6.3.1 Fermented (non-alcoholic) cereal beverages shall not exceed total aflatoxin of 10 ppb and 5 ppb for aflatoxin B_1 when tested in accordance with TZS 799.

6.3.2 Fermented (non-alcoholic) cereal beverages shall not exceed total of 2 ppm fumonisins when tested in accordance with annex B.

7 Hygiene

7.1 Fermented (non-alcoholic) cereal beverages shall be produced and handled under hygienic conditions in accordance with TZS 109.

7.2 Fermented (non-alcoholic) cereal beverages shall comply with microbiological limits given in Table 3 when tested in accordance with the methods specified therein.

Table 3 – Microbiological limits in fermented (non-alcoholic) cereal beverages

S/No	Microorganisms	Maximum limit	Method of Test
1	Total viable count, CFU/ml, max.	$1*10^2$	TZS 118-2
2	*Escherichia. coli*, per ml	Absent	TZS 730-2
3	*Salmonella* per 25 ml	Absent	TZS 122-1
4	Yeasts and moulds, CFU/ml, max.	$2 * 10^1$	TZS 2426-1

8 Sampling and test methods

8.1 Sampling

Sampling shall be done in accordance with Annex C.

8.2 Test methods

Testing shall be in accordance with the respective tables of this Tanzania Standard.

9. Packaging, Marking and Labelling

9.1 Packaging

Fermented (non-alcoholic) cereal beverages shall be packaged in food grade material that ensures the integrity and safety of the product.

9.2 Marking and Labelling

9.2.1 The product shall be marked and labelled in accordance with TZS 538. In addition to the requirements given in TZS 538, fermented (non-alcoholic) cereal beverages shall be legibly and indelibly labelled with the following information:

a) name of the product, “Fermented (non-alcoholic) cereal beverage”;

b) Brand or trade name of the product; if any

c) lot / batch identification number;

d) date of minimum durability (expiry date)

e) A declaration of common name of any additive used;

f) List of ingredients in descending orders of proportion;

g) manufacture date;

h) name and physical address of manufacturer;

i) The net content by volume in SI Units; and

j) country of origin

9.2.2 The language on the label shall be “Kiswahili” or “Kiswahili and English”. Any other language may be used depending on the designated market.

9.2.3 The containers may also be marked with the TBS standards mark of quality.

NOTE – The TBS standards mark of quality may be used by the manufacturers only under license from TBS. Particulars of conditions under which the licenses are granted may be obtained from TBS

9.2.4 Nutritional labelling

Nutritional labelling, nutrition and health claims may be made in accordance with TZS 481 and TZS 550.

Annex A
(normative)

Method for determination of total solids content

A.1 Weigh a suitable size evaporating dish and record the mass m_1.

A.2 By means of a measuring cylinder, transfer approximately 25 mL of the test sample to the evaporating dish.

A.3 Weigh the dish and the test specimen and record the mass m2.

A.4 Heat the dish on a boiling water bath until all the liquid has evaporated, then transfer the dish to an air oven, and dry for 30 min at 103 °C ± 1 °C.

A.5 Cool in a desiccator.

A.6 Reweigh the dish and the dried test specimen (residue) and record the mass m3. Calculate the total solids content as follows:

$$\text{Total solids content, \% } (m/m) = \frac{m_3 - m_1}{m_2 - m_1} \times 100$$

where

m_1 is the mass, in grams, of the dish;

m_2 is the mass, in grams, of the dish and specimen; and

m_3 is the mass, in grams, of the dish and dried specimen.

Annex B
(normative)

Determination of fumonisins B1 and B2 in fermented (non-alcoholic) cereal beverages by liquid chromatography with immune-affinity column cleanup

Applicability – It is applicable to determination of fumonisins B_1 in corn at total levels from 0.5 µg/g to 2 µg/g

Caution

Fumonisins are nephrotoxic, hepatotoxic, and carcinogenic to rats and mice, however effects on humans are not fully known. Wear protective gloves to reduce skin contact with extracts. Laboratory spills should be cleaned up by washing with a 5 % dilution of commercial bleach (sodium hypochlorite) followed by water.

A.1 Principle

Fumonisins are extracted from fermented (non-alcoholic) cereal beverages with methanol–acetonitrile–water (25 + 25 + 50, v/v/v), the filtered extract is cleaned up by an immunoaffinity column, and the fumonisins are eluted with methanol. The eluate is evaporated just to dryness, and the residue is dissolved in acetonitrile–water (50 + 50, v/v). O- Phthaldialdehyde and 2-mercaptoethanol is added to form fluorescent fumonisin derivatives, which are separated by reversed phase liquid chromatography (LC) with fluorescence detection.

A.2 Reagents

a) Methanol, LC grade.
b) Acetonitrile, LC grade.
c) O-Phthaldialdehyde (OPA), CAS 643-79-8.
d) 2-Mercaptoethanol (MCE), CAS 60-24-2.
e) Sodium dihydrogen phosphate solution, 0.1 M. Dissolve 15.6 g $NaH2PO4{\cdot}2H_2O$ in water and dilute to 1 L.
f) Sodium tetraborate solution, 0.1 M. Dissolve 3.8 g Na2B4O7·10H2O in water and dilute to 100 mL.
g) Hydrochloric acid, 2 M. Dilute HCl (12M) 1+5 with water.
h) Extraction solvent, Acetonitrile–methanol–water (25 + 25+ 50, v/v/v).
i) Acetonitrile–water, 50 + 50, v/v.
j) Phosphate-buffered saline (PBS), Dissolve 8.0 g NaCl, 1.2 g anhydrous Na 2HPO4, 0.2 g KH2PO4, and 0.2 g KCl in approximately 990 mL water. Adjust pH to 7.0 with 2M HCl, and dilute to1 L. Phosphate-buffered saline tablets can also be used.
k) Immunoaffinity columns, Specific for fumonisin cleanup with 100 % cross reactivity for both FB1 and FB2. The column must have a total capacity of $ 10 µg fumonisins B1 and B2 and should give a recovery of $ 90 % when a calibrant solution of fumonisins B1 and B2 in methanol–PBS containing 5µg fumonisins is applied. Follow the manufacturer's instructions for the type of column used.
l) LC mobile phase, Methanol, 0.1 M NaH2PO4 (77 + 23, v/v), adjusted to pH 3.35 with H3PO4. Filter mobile phase through 0.45 µm membrane, and pump at 1 mL/min flow rate. Adjust com- position to conform with individual LC column characteristics.
m) OPA reagent, dissolve 40 mg OPA in 1 mL methanol, and dilute with 5 mL 0.1 M Na2B4O7 solution. Add 50 µL MCE and mix. Store in the dark for up to 1 week at room temperature in a capped amber vial.
n) Fumonisins B1 and B2, Crystalline form, purity of >95 %.
o) Fumonisin stock solution for LC, Prepare calibrant solution containing fumonisins B1 and B2 in acetonitrile–water (50 + 50, v/v) at concentration of 100 µg/mL for FB1 and 50 µg/mL for FB2. Fumonisin calibrant solution is stable up to 6 months when stored at 4 °C. Pipet 500 µL fumonisin calibrant solution into a 5-mL calibrated volumetric flask. Dilute to volume with acetonitrile–water (50 + 50,v/v), and shake well to obtain stock solution containing FB1 at10 ng/µL and FB2 at 5 ng/µL.
p) Fumonisin working calibrant solutions for LC, Prepare 4 LC calibrant solutions in separate 5-mL volumetric flasks according to table 2001.04C. Dilute contents of each flask to volume (5 mL) with acetonitrile–water (50 + 50, v/v).

A.3 Apparatus

a) Centrifuge bottle, Plastic, 250 mL, with screw cap
b) Centrifuge, Operating up to 2500 ×g
c) Filter papers, Whatman No. 4, 12 cm.

d) Glass microfiber filters, Whatman GF/A, 9 cm
e) Reservoir, 25 mL with Luer tip connector for immunoaffinity column
f) Calibrated microliter syringe or microliter pipet, 25 to 1000 µL
g) Vacuum manifold, To accommodate immunoaffinity columns
h) Liquid chromatograph, LC pump delivering 1 mL/min constant flow rate and with injection system calibrated to deliver 20 µL; and data system
i) LC column, Stainless steel (150×4.6 mm id), packed with5 µm C18 deactivated reversed-phase material, preceded by corresponding reversed-phase guard column or guard filter (0.5 µm) porosity.
j) Fluorescence detector, Fitted with flow cell and set at 335 nm (excitation) and 440 nm (emission).

A.4 Extraction

Permit materials to reach room temperature before removing test portion. Weigh, to nearest 0.1 g, 20 g test portion of fermented (non-alcoholic) cereal beverages into 250 mL centrifuge bottle, and add 50 mL extraction solvent, 2(h). Cover centrifuge bottle, and shake bottle for 20 min with orbital shaker. Centrifuge for 10 min at 2500×g, and filter supernatant through filter paper, A.3 (c), avoiding transfer of solid ma-terial on filter. Again extract remaining solid material by adding 50 mL extraction solvent, 2 (h), to centrifuge bottle and shaking bottle for 20 min. Centrifuge for 10 min at 2500 ×g, and filter extract through the same filter paper. Collect and combine the 2 filtrates, and pipet 10 mL filtrate into 100 mL flask. Add 40 mL PBS, 2 (j), and mix well. Filter diluted extract through microfiber filter, A.3 (C) or (d), and collect 10 mL filtrate (equivalent to 0.4 g test portion) for cleanup through immune-affinity column.

A.5 Immunoaffinity column cleanup

Follow manufacturer's instructions for the type of column used. Remove top cap from column, and connect column with reservoir. Remove end cap from column and attach column to vacuum manifold. Pipet 10 mL filtrate into reservoir. Let filtrate flow through column at ca 1–2 drops/s and discard eluate. Wash column with 10 mL PBS, 2(j), at rate of 1–2 drops/s until air comes through column. Place 4 mL vial under column. Elute fumonisin with 1.5 mL LC grade methanol at 1 drop/s, and collect fumonisin in vial. Evaporate eluate just to dryness under stream of N at ca 60 °C. Retain dried residue at ca 4 °C for derivatization and LC analysis

A.6 Calibration curve

Prepare calibration curves, using working calibrant solutions 2 (p). These solutions cover the range of 0.025–2.000 µg/g for FB1 and the range of 0.0125–1.000 µg/g for FB2. Prepare calibration curves, before LC analysis, according to table 2001.04C, and check plots for linearity. If curve is not linear, repeat derivatization following instructions carefully and/or reduce the range of the calibrants.

A.7 Derivatization and LC analysis

Re-dissolve purified residue in 200 µL acetonitrile–water (50 + 50, v/v), B(i). Transfer 50µL aliquots of extract or standards to bottom of 1 mL test tube, and add 50µL OPA reagent, B(m). Mix solution for 30 s with vortex mixer, and inject 20 µL derivatized solution (equivalent to 20 mg matrix) into LC system exactly 3 min after add-ing OPA reagent. With the described LC mobile phase, B(l), and column, C(i), satisfactory (baseline) resolution of FB1–OPA and FB2–OPA must be obtained, with expected retention times at ca 6 and 15 min, respectively. If fumonisin content of derivatized extract is higher than calibration range, dilute purified extract with acetonitrile–water (50 + 50, v/v), B(i), derivatize with OPA reagent, and repeat LC analysis.

A.1Quantification of fumonisin B1and B2

Quantify FB1 and FB2 by measuring peak area (or peak height) at retention time of each fumonisin and comparing measured value with corresponding calibration curve.

From calibration curves determine amounts of FB1 and FB2 (in µg) in aliquot of test solution injected into LC column.

Separately calculate concentrations (CFB) of FB1 and FB2 in micrograms per gram (µg/g), as follows:

$$CFB = \frac{10^3 M_A \times 1}{10^3 M_B} \quad \frac{M_A}{20}$$

where

MA is mass of FB1 or FB2 (in µg) in aliquot of test solution injected on column, as determined from calibration curve,
MB is mass of matrix (in mg) injected on column (20 mg), obtained as follows:

$$\frac{20 \text{ g (Extraction step)}}{100 \text{ Ml}} \times \frac{10 \text{ mL (dilution)}}{50 \text{ Ml}} \times \frac{10 \text{ mL (purified aliquot)}}{1 \text{ (reconstitution volume)}} \times \frac{0.05 \text{ mL (derivatization)}}{0.2 \text{ mL}} \times \frac{}{0.1 \text{ mL}}$$

Where
0.02 mL is the injected aliquot
And 10^{-3} is a factor to convert M_A and M_B from ng to µg and mg to g, respectively.

Annex C
(normative)

Sampling

B.1 Definitions

B.1.1 Lot

Collection of primary containers or units of the same size, type, and style manufactured or packed under similar conditions and handled as a single unit of trade.

B.1.2 Lot size

Number of primary containers or units in the lot.

B.1.3 Sample size

Total number of sample units drawn for examination from a lot.

B.1.4 Sample unit

Portion of the contents of a container, or a composite mixture of product from small containers that is sufficient for the examination or testing as a single unit. For fill of container, the sample unit shall be the entire contents of the container

B.2 Sampling plans

B.2.1 Samples shall be randomly/representatively selected from each lot separately and tested for ascertaining their conformity to the requirements of relevant specifications.

B.2.2 Sampling shall be done in accordance with the plan given in Table B.1.

Table B.1 — Sampling plan

Lot size	**Sample size** n
Net weight equal to or less than 1 kg (2.2 lb)	
4 800 or less	13
4 801 to 24 000	21
24 001 to 48 000	29
48 001 to 84 000	48
84 001 to 144 000	84
144 001 to 240 000	126
Over 240 000	200
Net weight greater than 1 kg (2.2 lb) but not more than 4.5 kg (10 lb)	
2 400 or less	13
2 401 to 15 000	21

15 001 to 24 000	29
24 001 to 42 000	48
42 001 to 72, 000	84
72 001 to 120 000	126
Over 120 000	200
Net weight greater than 4.5 kg (10 lb)	
600 or less	13
601 to 2 000	21
2 001 to 7 200	29
7 201 to 15 000	48
15 001 to 24 000	84
24 001 to 42 000	126
Over 42 000	200
NOTE n is number of primary containers in sample.	