ICS 67.220.20

DMS 1257:2023

Second edition

DRAFT MALAWI STANDARD

Baker’s yeast – Specification

NOTE: This is a draft proposal and it shall neither be used nor regarded as a Malawi Standard

ICS 67.220.20

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Baker's yeast – Specification

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FOREWORD

This draft Malawi standard was prepared by MBS/TC 19, the Technical Committee on *Bread and confectioneries,* to provide requirements for baker's yeast. It is the revision of MS 1257:2016, *Baker's yeast – Specification*.

In preparing this draft Malawi standard reference was made to the following standard:

East African Standard, EAS 997:2019, *Baker's yeast – Specification*.

Acknowledgement is made for the use of the information.

TECHNICAL COMMITTEE

This draft Malawi standard was prepared by MBS/TC 19, the Technical Committee on *Bread and confectioneries*, and the following companies, organizations and institutions were consulted:

Bakelines Limited;

Bakeman's Confectioneries Limited;

Bakhresa Malawi;

Blantyre City Council;

Bread Talk;

Bvumbwe Agricultural Research Station;

Competition and Fair Trading Commission;

Consumers Association of Malawi;

H.M.S Food and Grains Limited;

International Potato Centre Malawi;

Kachere Bakery;

Lilongwe University of Agriculture and Natural Resources;

Malawi University of Business and Applied Sciences;

Mega Bakers;

Ministry of Health – Department of Nutrition, HIV and AIDS;

Ministry of Trade and Industry;

National Fortification Alliance;

Rab Processors;

Shoprite Trading Limited;

Tehilah Bakery;

University of Malawi; and

Universal Industries.

NOTICE

This standard shall be reviewed every five years, or earlier whenever it is necessary, in order to keep abreast of progress. Comments are welcome and shall be considered when the standard is being reviewed.

DRAFT MALAWI STANDARD

Baker's yeast – Specification

1 SCOPE

This draft Malawi standard specifies requirements, sampling and test methods for baker's yeast.

2 NORMATIVE REFERENCES

The following standards contain provisions which, through reference in this text, constitute provisions of this draft proposal. All standards are subject to revision and, since any reference to a standard is deemed to be a reference to the latest edition of the standard, parties to agreements based on this draft Malawi standard are encouraged to take steps to ensure the use of the most recent editions of the standards indicated below. Information on currently valid national and international standards can be obtained from the Malawi Bureau of Standards.

MS 19: *Labelling of prepacked foods – General standard;*

MS 21: *Food and food processing units – Code of hygienic conditions;*

MS 30: *Fortified wheat flour – Specification;*

CAC/GL 50: *General guidelines on sampling;*

ISO 4832; *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of Coliforms – Colony-count technique;*

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 6888-1: *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Part 1: Technique using Baird-Parker agar medium;*

ISO 6888-2*: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Part 2: Technique using rabbit plasma fibrinogen agar medium;*

ISO 6888-3: *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Part 3: Detection and MPN technique for low numbers;*

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

ISO 15914: *Animal feeding stuffs – Enzymatic determination of total starch content.*

3 TERMS AND DEFINITIONS

For the purposes of this draft Malawi standard, the following terms and definitions shall apply:

3.1
baker's yeast
cells of one or more strains of the yeast *Saccharomyces cerevisiae* that aid the fermentation and aromatic activity of fermented doughs

3.2
Fresh Baker's Yeast (FBY)
baker's yeast consisting of living cells of *Saccharomyces cerevisiae*

3.3
Dry Baker's Yeast (DBY)
baker's yeast consisting of living but inactive cells of *Saccharomyces cerevisiae*

4 TYPES OF BAKER'S YEAST

Baker's yeast shall be categorised in two types as follows:

4.1 Fresh Baker's Yeast (FBY)

FBY may be in three major forms:

4.1.1 Block or compressed yeast

This shall be in the form of a block. The texture or consistency shall be either high plasticity (kneadable, deformation possible without breakage) or friable/crumbly (blocks easily broken into small pieces).

4.1.2 Granulated yeast

This shall be in the form of small granules.

4.1.3 Liquid yeast

This shall be a liquid suspension of yeast cells in water with a cream-like viscosity.

4.2 Dry Baker's Yeast (DBY)

DBY may be in the following forms:

4.2.1 Active dry yeast

Yeast that requires reactivation by rehydration using warm water (38 °C – 45 °C) prior to use. It may be of spheroid particles, 0.2 – 3 mm in diameter.

4.2.2 Instant dry yeast

Yeast dried in a way that rehydration is not necessary to facilitate reactivation. It consists of porous cylindrical yeast particles with an approximate diameter of 0.5 mm and length up to a few millimetres.

5 ESSENTIAL COMPOSITION AND QUALITY FACTORS

5.1 General quality requirements

Baker's yeast shall:

5.1.1 Be ivory in colour;

5.1.2 Have an odour typical of yeast;

5.1.3 Be free of extraneous materials;

5.1.4 Not be slimy or mouldy; and

5.1.5 Not show any signs of deterioration or decomposition.

5.2 Specific compositional requirements

Baker's yeast shall comply with the compositional requirements given in **Table 1** when tested in accordance with the test methods specified therein.

Table 1 – Compositional requirements for baker's yeast

S/N	Characteristic	Requirement		Test method
		Fresh baker's yeast (FBY)	Dry baker's yeast (DBY)	
1	Moisture, % (m/m), max	73	8	Annex A
2	Edible starch, % (m/m), max	7	10	ISO 15914
3	Dispersibility in water	No yeast cell deposits	No yeast cell deposits	Annex B
4	Fermenting power, ml, min	1000	350	Annex C
5	Dough-raising capacity	To satisfy the test	To satisfy the test	Annex D

5.3 Microbiological limits

Baker's yeast shall comply with the microbial limits given in **Table 2** when tested in accordance with the test methods specified therein.

Table 2 – Microbiological limits for baker's yeast

S/N	Characteristic	Maximum limits		Test method
		Fresh Baker's Yeast (FBY)	Dry Baker's Yeast (DBY)	
1	*Coliform count*, cfu/g	10	50	ISO 4832
2	*Escherichia coli*, MPN/g	Absent	Absent	ISO 7251
3	*Salmonella* in 25 g	Absent	Absent	ISO 6579
4	*Staphylococcus aureus,* cfu/g	10	10	ISO 6888
5	Rope spore count, cfu/g	10	100	Annex E
6	Bacterial flora, other than yeast, millions per gram (on dry basis)	0.75	8.0	Annex F

6 HYGIENE

Yeast shall be manufactured, packaged and stored under hygienic conditions in premises conforming to the requirements prescribed in MS 21.

7 PACKAGING AND STORAGE

7.1 Fresh Baker's Yeast

The FBY blocks shall be wrapped or packed in clean waxed paper or any other suitable food grade wrapping material or non-toxic wrappers to preserve freshness and to prevent undue deterioration during storage.

The yeast blocks shall be stored at the temperatures of between 1 to 6^0 C.

7.2 Dry Baker's Yeast

The DBY shall be packed in clean, sound and suitable airtight containers preferably tin containers such a manner as to prevent the absorption of moisture and to prevent undue deterioration during storage.

The yeast shall be stored in a cool and dry place at a temperature of not more than 25^0 C.

8 LABELLING

In addition to the labelling requirements stipulated in MS 19, the packages shall be legibly and indelibly marked with the following information:

8.1 Name of the product shall be “Baker’s Yeast”. Type of the product may be indicated in close proximity to the name of the product e.g. “Baker’s Yeast, Granulated”, “Instant Baker’s Yeast”, “Granulated Baker’s Yeast”, etc.;

8.2 Name and physical address of the processor/packer/importer;

8.3 Date of manufacture;

8.4 Expiry date;

8.5 Batch or code number;

8.6 Net weight of the product in metric units;

8.7 List of ingredients in descending order by quantity when used;

8.8 Country of origin;

8.9 Storage instructions; and

9.0 Declaration 'Contains edible starch', when edible starch is added.

10 SAMPLING AND TEST METHODS

Representative samples shall be drawn in accordance to the procedure outlined in **Annex G** and tested using appropriate standard methods declared in this draft Malawi standard.

Annex A
(Normative)

DETERMINATION OF MOISTURE

A.1 APPARATUS

A.1.1 Dish, with a cover, made of glass or aluminium, about 25 mm in diameter

A.1.2 Glass stirring rod, approximately 60 mm long, with a flattened end.

A.2 REAGENT

Alcohol, Ethyl alcohol or **rectified spirit.**

A.3 PROCEDURE

Weigh the dish with the cover and stirring rod (*M*). Transfer to this, add about 10 g of dry yeast or 2.5 g of fresh yeast and weigh accurately to the nearest milligram (M_1). Remove the cover of the dish and add 5 ml of alcohol. Mix thoroughly using the stirring rod and leave the stirring rod in the weighing dish. Place the cover on the dish and dry at 105 ± 1 °C for 4 h for fresh baker's yeast and 6 h for dry baker's yeast. Cool the dish in a desiccator and weigh (M_2).

A.4 CALCULATION

The moisture content, expressed as percent by mass, shall be calculated as follows:

$$\frac{100x\ (M_1 - M_2\ )}{M_1 - M}$$

Where,

M is the mass, in grams, of the dish, its cover and the stirring rod;

M_1 is the mass, in grams, of the dish, its cover and the stirring rod with the sample before drying, and,

M_2 is the mass, in grams, of the dish, its cover and the stirring rod with the sample after drying.

Annex B
(Normative)

TEST FOR DISPERSIBILITY IN WATER

B.1 APPARATUS

B.1.1 Beaker, 400 ml.

B.1.2 Measuring cylinder, 100 ml and 1000ml.

B.1.3 Thermometer.

B.2 REAGENT

B.2.1 Distilled water.

B. 3 PROCEDURE

B. 3.1 Dry baker's yeast

Weigh 5 g of dry baker's yeast into a 400 ml beaker and add 50 ml of distilled water at 40 °C. Leave the product undisturbed for 5 min and thereafter, stir for 2 min. To a one-litre graduated cylinder, add 900 ml of distilled water at 40 °C. Pour the slurry in the beaker into the water in the graduated cylinder. Wash the beaker with 50 ml of distilled water, pour it into the cylinder and leave it undisturbed for 5 min. Check for any deposits at the bottom of the cylinder. If no deposits appear at the bottom of the cylinder, the material shall be considered to have passed the test.

B.3.2 Fresh baker's yeast

Weigh 20 g of fresh baker's yeast into a 400 ml beaker and add 50 ml of distilled water at 40 °C. Leave the product undisturbed for 5 min and thereafter, stir for 2 min. To a one-litre graduated cylinder, add 900 ml of distilled water at 30 °C. Pour the slurry in the beaker into the water in the graduated cylinder. Wash the beaker with 50 ml of distilled water, pour it into the cylinder and leave it undisturbed for 5 min. Check for any deposits at the bottom of the cylinder. If no deposits appear at the bottom of the cylinder, the material shall be considered to have passed the test.

Note: If starch was added to the yeast, it may form a sediment, which may contain a few yeast cells.

Annex C
(Normative)

DETERMINATION OF FERMENTING POWER

C.1 APPARATUS

C.1.1 Fermentometer. The assembly of the apparatus is illustrated in **Figure 1.** It consists of a 250 ml flat-bottomed flask (**A**), whose mouth is fitted with a ground-glass joint having a glass delivery tube bent at right angle. It is connected to a three-way T-shaped stop-cock (**B**) which in turn is fitted on a 100 ml graduated tube (**D**) of the manometer. **E** is the manometer reservoir of 250 ml capacity. **I** is the iron stand. **D** and **E** are connected by a PVC tube **F. G** is a water bath.

C.1.2 Barometer.

C.1.3 Thermometer.

C.2 REAGENTS

C.2.1 Sugar phosphate mixture. Grind and mix thoroughly 400 g of sucrose, 25 g of diammonium hydrogen phosphate [$(NH4)_2 HPO_4$] and 25 g of dipotassium hydrogen phosphate ($K_2 HPO_4$).

C.2.2 Calcium sulphate solution. Dilute 30 g of saturated calcium sulphate solution ($CaSO_4 .2H_2 O$) with 70 g of distilled water.

C.2.3 Manometer solution. Weigh 200 g of anhydrous calcium chloride and 10 g of cupric chloride and dissolve in distilled water. Add a little hydrochloric acid so that the final pH after making up the solution to two litres does not exceed 5.0.

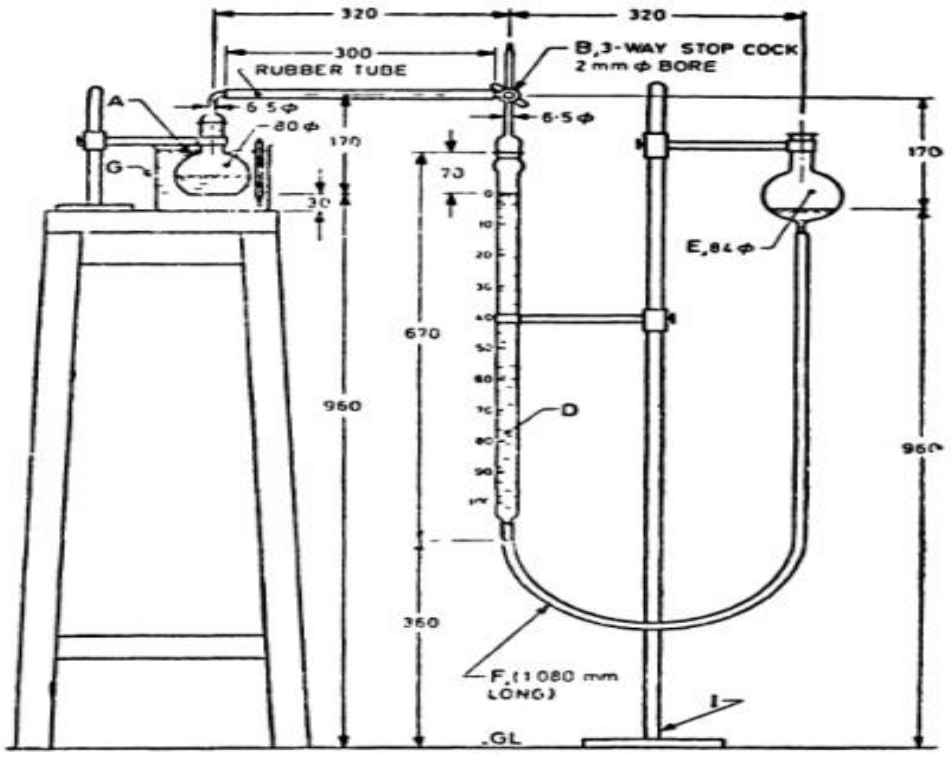

Figure 1: Fermentometer apparatus

C.3 PROCEDURE

C.3.1 Mix 6.75 g of the sugar phosphate mixture with 75 ml of the calcium sulphate solution in the flask. Add to it, 3.67 g of fresh baker's yeast or 0.893 g of dry baker's yeast. Stir well to disperse the yeast. Keep the flask in the water bath at 30°C throughout the experiment. Bring the three-way T-shaped stop-cock of the manometer into a position which allows displacement of initial air (by the carbon dioxide evolved) to escape to the atmosphere without displacement of the manometer fluid. This displacement is allowed for the first 13 min after which the stop-cock position is altered to allow the carbon dioxide evolved to enter the manometer and bring about the displacement of the manometer fluid. Shake the contents of the flask every 10 min.

C.3.2 While taking the reading of the gas evolved, the level of the fluid in the manometer shall be adjusted by sliding the reservoir arm of the manometer and the volume of gas evolved at this pressure (which will now be equal to the atmospheric pressure) shall be recorded.

C.3.3 As soon as the reading is taken, the initial gas formed which has just been measured, is allowed to escape into the atmosphere by operating the three-way stop-cock and the stop-cock position is again adjusted to take the second reading. For fresh baker's yeast, readings should be taken every 10 minutes and for dry baker's yeast, readings shall be taken every 30 min. In both the cases, readings shall be taken for 3 h.

C.3.4 The room temperature and the atmospheric pressure shall also be noted during the course of the experiment. The readings are recorded in a tabulated form (see Table C.1) and the total volume of gas produced is calculated and corrected at 101 kPa pressure and 20°C temperature by the formula given in C.4.

Table C.1 – Recording carbon dioxide evolved every 10/30 min

Time	**Volume of CO_2 evolved (ml)**	**Room temperature (^{0} C)**	**Atmospheric pressure (mmHg)**	**Corrected volume (ml)**
10:00 am	.....	.....	.....	.....
10:10 am	.....	.....	.....	.....
10:20 am	.....	.....	.....	.....
10:30 am	.....	.....	.....	.....
10:40 am	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
1:00 pm	.....	.....	.....	.....
	Total volume	Average temperature	Average pressure	

C.4 CALCULATION

The fermenting power, expressed as corrected volume in millilitres, shall be expressed as follows:

$$Corrected\ Volume\ = \frac{\text{Observed volume x Observed average pressure x293}}{\text{760 x273+Average room temperature}}$$

The mass of carbon dioxide evolved, expressed in grams, may be calculated from this corrected volume as follows:

$$mass\ of\ carbondioxide\ evolved = \frac{44 \text{ x V}}{22400}$$

Where,

V is the corrected volume of carbon dioxide evolved.

Annex D
(Normative)

DETERMINATION OF DOUGH RAISING CAPACITY

D.1 APPARATUS

D.1.1 Beaker, 500 ml.

D.1.2 Measuring cylinder, 100 ml.

D.1.2 Calibrated weighing balance.

D.2 MATERIALS

D.2.1 Wheat flour conforming to MS 30

D.2.2 Sucrose.

D.2 PROCEDURE

D.2.1 Mix 4.0 g of fresh baker's yeast or 1.0 g of dry baker's yeast with 100 g of wheat flour. Add 1.0 to 1.5 g of sucrose and a suitable quantity of water (about 55 ml). Knead well. Press the resulting dough into a glass beaker. Note the level of the dough by means of a scale, from the bottom of the beaker. Keep it covered for one hour at 27°C. At the end of this period, note the level again.

D.2.2 The product shall be deemed to have satisfied the test if the rise in level is at least 80 percent of the original for dry baker’s yeast and 110 % for fresh baker’s yeast.

Annex E
(Normative)

DETERMINATION OF BACTERIAL ROPE SPORE COUNT

E.1 APPARATUS

E.1.1 Flask, 100 ml.

E.1.2 Conical flask, 250 ml.

E.1.3 Water bath.

E.1.4 Pipette, 1 ml and 10 ml.

E.1.5 Petri dishes.

E.2 REAGENTS

E.2.1 Sterilised peptone water, 0·1 %

E.2.2 Tryptone glucose extract (TGE) Agar composed of:

E.2.2.1 Tryptone 5.0 g;

E.2.2.2 Agar, bacteriological grade, 15.0 g. Granulated or chopped shreds, practically free from thermophilic bacteria shall be used;

E.2.2.3 Yeast extract 2.5 g;

E.2.2.4 Distilled water, one litre;

E.2.2.5 Sodium chloride 6.5 g;

E.2.2.6 Glucose (dextrose) 1.0 g; and

E.2.2.7 Final pH of 7.0 ± 0.1.

E.2 PROCEDURE

E.2.1 Weigh 22 g of wheat flour in a suitable sanitised container and transfer to a conical flask containing 100 ml of sterile 0.1 % peptone water and sterile sand or glass beads. Blend on a shaker for about two minutes. Dilute the blended mixture further, 1:10, 1:100, 1:1 000, 1:10 000, etc., by dilution technique, using sterile peptone water.

E.2.2 Prepare tryptone glucose extract (TGE) agar; 100 ml per 250 ml conical flask. Prepare one additional flask of medium to serve as sterility control. Sterilise at 121°C for 15 min and then cool to 45°C in a waterbath. Pipette volumes of the blended mixture into a series of TGE agar flasks while they are held in the waterbath; 10 ml into the first, 1 ml into the second and 1 ml of each dilution into the third, fourth and fifth TGE flask and so on. Gently agitate the flasks to disperse the blended mixture throughout the medium.

E.2.3 Transfer the flasks without delay to a waterbath adjusted to 65°C to 90°C and hold for 30 min with gentle shaking occasionally to assist heat distribution. After 30 min of heat treatment, cool the flasks to about 45°C without allowing the agar to gelatinise. Pour 100 ml of the medium into each flask representing the product and sterility control into a set of five sterile petri dishes in approximately equal volumes of about 20 ml per plate. When agar has solidified, invert the plates and incubate at 35°C for 48 h.

E.2.4 Count the surface and sub-surface colonies. The sum of the colonies on the set of five plates poured from TGE agar, containing 10 ml of the blended mixture represents the number of aerobic and mesophilic spores per gram of the product. Similarly, 1 ml of the blended and 1 ml of each dilution are equal to 0.01, 0.001, 0.000 1 and 0.000 01 of the number of spores per gram and shall be multiplied by the respective dilution factor. Generally, the set of plates showing about 30 to 60 colonies per plate are to be chosen for the counting purposes.

E.3 PRECAUTIONS AND LIMITATIONS

E.3.1 The procedure permits enumeration of aerobic and mesophilic spores in food samples containing relatively higher number of spores by higher dilution of the samples prior to heat treatment.

E.3.2 Certain thermophilic strains may also be indicated in this method in which case a separate enumeration method for thermophiles may be adopted and their numbers subtracted from the spore count

Annex F
(Normative)

DETERMINATION OF BACTERIAL FLORA OTHER THAN YEAST

F.1. METHOD 1

F.1.1 APPARATUS

F.1.1.1 Sterile weighing scoop – with counterweight.

F.1.1.2 Sterile bacteriological transfer pipettes – accurancy graduated and with non-absorbent cotton plug in the sucking end.

F.1.1.3 Sterile dilution flasks – made of heat resistant glass.

F.1.1.4 Sterile petri dishes – outside diameter 100 mm, with inside diameter 90 mm and depth 16 mm. The exterior and interior surfaces of the bottom shall be flat and free from bubbles, scratches or other defects.

F.1.1.5 Sterile bacteriological tubes – of 25 ml capacity with a mark at the 10 ml level and fitted with a non-absorbent cotton plug.

F.1.2 REAGENTS

F.1.2.1 Sterile citric acid solution – prepare an approximately 10% (m/v) solution of citric acid in water and sterilise it in an autoclave at 120^0 C for 15 minutes.

F.1.2.2 Dextrose agar medium – having the following composition: peptone 20 g, dextrose 40 g, agar 25 g and distilled water 1000 ml.

F.1.2.2.1 Preparation of the dextrose agar medium

Steam the ingredients to dissolve and filter through chardin filter paper at 60^0 C, sterilise this in an autoclave at 120^0 C for 15 minutes. Cool to about 90^0 C and adjust to pH 3.5 with the sterile citric acid solution (**F.1.2.1**)**.** Pour the medium into each of the sterile bacteriological tubes (**F.1.1.5**) up to the 10ml mark (do not sterilise again).

F.1.2.3 Nutrient agar medium – having the following composition: beef extract 3 g, peptone 6 g, agar 25 g and distilled water 1000 ml.

F.1.2.3.1 Preparation of nutrient agar medium

Steam the ingredients to dissolve. Adjust to pH 6.2 to 7.2. Boil the medium and filter through chardin filter paper at 60^0 C. Pour the medium into the bacteriological tubes up to 10 ml mark and sterilize in an autoclave at 120^0 C for 15 minutes.

F.1.2.4 Ringer's solution (full strength)- having the following composition: sodium chloride (NaCl) 9.0 g, potassium chloride (KCl) 0.42 g, calcium chloride (CaCl) 0.24 g, sodium bicarbonate ($NaHCO_3$) 0.20 g and distilled water 1000 ml.

F.1.2.4.1 Preparation of ringer's solution

F.1.2.4.1.1 Dissolve the ingredients and sterilize in an autoclave at 120^0 C for 15 minutes.

F.1.2.4.1.2 Dilute the Ringer's solution to four times with sterile distilled water before use.

F.1.3 PROCEDURE FOR DETERMINING YEAST CELL COUNT

F.1.3.1 Dilution – Prepare a dilution of the material in the Ringer's solution **F.1.2.4.1.2** so that 1ml of the diluted suspension when plated as described in **F.1.3.2** give between 30 and 300 colonies . Add 1 ml of this dilution to the sterile petri dishes. Use at least five dilutions.

F.1.3.2 Pouring of plates – melt the dextrose agar medium in the bacteriological test tubes **F.1.2.2.1** and immediately cool to about 45^0C (prolonged heating at higher temperature hydrolyses the agar and affects the gelling properties of the medium). Pour this melted medium into the petri dishes and mix by rotating and tilting the dish without splashing over the edge. Allow to solidify.

F.1.3.3 Incubation – Invert the petri dishes and incubate at 30^0C for 3 days.

F.1.3.4 Counting – count the yeast colonies with the aid of magnification under uniform and properly controlled illumination. Count only those plates with 30 to 300 colonies.

F.1.4 PROCEDURE FOR DETERMINING TOTAL PLATE COUNT

F.1.4.1 Dilution – prepare a dilution of the material in the Ringer's solution **F.1.2.4.1.2** so that 1ml of the diluted suspension when plated as described in **F.1.4.2** give between 30 and 300 colonies . Add 1 ml of this dilution to the sterile petri dishes. Use at least five dilutions.

F.1.4.2 Pouring of plates – melt the nutrient agar medium in the bacteriological test tubes **F.1.2.3.1** and immediately cool to about 450^0C. Pour this melted medium into the petri dishes and mix by rotating and tilting the dish without splashing over the edge. Allow to solidify.

F.1.4.3 Incubation – Invert the petri dishes and incubate at 37^0C for 2 days.

F.1.4.4 Counting – count the colonies with the aid of magnification under uniform and properly controlled illumination. Count only those plates with 30 to 300 colonies.

F.1.5 CALCULATION

F.1.5.1 Calculation for yeast cell count

F.1.5.1.1 Complete the average yeast cell count per gram from the dilution used.

F.1.5.1.2 Yeast cell count per gram (on dry basis):

$$y_c = \frac{100xc_w}{100-M}$$

Where,

c_w is the average yeast cell count per gram **F.1.5.1.1**; and

M is the moisture, percent by mass , as determined in **annex A.**

F.1.5.2 Calculation for bacterial flora other than yeasts

F.1.5.2.1 Complete the average total plate count per gram from the dilutions used.

F.1.5.2.2 Total plate count per gram (on dry basis):

$$Tc = \frac{100 \text{ x } Tw}{100-M}$$

Where,

Tw is theaverage total plate count per gram, **F.1.5.2.1;** and

M is the moisture, percent by mass , as determined in **annex A.**

F.1.5.2.3 Bacterial flora other than yeasts, count per gram (on dry basis) = $T_C - Y_C$

Where,

T_c is the total plate count per gram (on dry basis); and

Y_c is the Yeast cell count per gram (on dry basis).

F.2 METHOD 2

F.2.1 PRINCIPLE

The growth of yeast cells is inhibited by addition of chemicals such as nystatin or cycloheximide (actidione) and the material in a suitable medium is incubated at 37^0 C for 48 hours. The bacterial flora other than yeast is then counted.

F.2.2 APPARATUS

Same as given from **F.1.1.1** to **F.1.1.5.**

F.2.3 REGAENTS

F.2.3.1 Nystatin or cycloheximide (actidione) solution – 0.22 % (m/v), sterilised at 36 kPa for 20 minutes.

F.2.3.2 Yeast suspension (m/v basis) – dilution 10^{-1} to 10^{-6} .

F.2.3.3 Nutrient agar and yeast extract medium.

F.2.3.3.1 Nutrient agar – having the following composition: Bacto beef extract 3.0 g, bacto peptone 5.0 g, sodium chloride 8.0 g, bacto agar 15.0 g, distilled water 1000 ml and pH 7.0.

F.2.3.3.2 Suspend nutrient agar in water (23 g/litre of water) and add 10 g of yeast extract. Heat in the boiling water bath to dissolve. Dispense the agar (12 ml) while hot into test tubes and cover with oxold caps. Sterilise by autoclaving at 100 kPa for 15 minutes.

F.2.4 PROCEDURE

F.2.4.1 In each petri dish, first pour 1ml of nystatin or cycloheximide solution (0.12 %, m/v), then pour 1 ml of the appropriate dilution of yeast suspension and finally one tube of nutrient agar medium **F.2.2.3.1** at a temperature of 45 to 46^0 C and mix by rotating and tilting the dishes without splashing over the edge. Allow to solidify.

F.2.4.2 When set, invert the petri dishes and incubate for 48 hours at 37^0 C.

F.2.4.3 Count each colony and multiply the total by the appropriate dilution used.

F.2.4.4 Compute average count per gram of bacterial flora other than yeast (N).

F.2.5 CALCULATION

Bacterial flora other than yeast, per gram (on dry basis) $= \frac{100 \text{ x N}}{100 \text{ X M}}$

Where,

N is the average count per gram of bacterial flora other than yeast; and

M is the moisture , percent by mass, as determined in **annex A.**

Annex G
(Normative)

SAMPLING OF BAKER'S YEAST

G.1 GENERAL REQUIREMENTS OF SAMPLING

In drawing, preaparing, storing and handling samples the following precautions and directions shall be observed.

G.1.1 The sampling instrument shall be clean and dry when used. When taking samples for microbiological examination, the sampling instrument shall be sterile.

G.1.2 Precuations shall be taken to protect the samples, the materials being sampled, the sampling instruments and the containers for samples, from adventitious contamination such as damp air, dust and soot.

G.1.3 The samples shall be placed in clean and dry glass containers. The sample containers shall be of such a size that they are almost completely filled by the sample. The sample container shall in addition be sterile when they are used for storing samples for microbiological examination.

G.1.4 Each container shall be sealed air tight after filling and marked with full details of sampling, batch number, name of the manufacturer and other important particulars of the consignment.

G.1.5 Fresh baker's yeast samples shall be refrigerated immediately at a temperature between 1 – 5^0 c and shall be tested within 24 hours. Dry baker's yeast shall be refrigerated immediately and tested within 48 hours.

G.1.6 Sampling shall be done by a person agreed to between the purchaser and the vendor and in the presence of the purchaser (or his representative) and the vendor (or his representative).

G.2 SCALE SAMPLING

G.2.1 Lot

All the containers in a single consignment of the material of same type and drawn from a single batch of manufacture shall constitute a lot. If the consignment is declared to consist of different batches of manufacture, containers of the same batch shall be separated and shall constitute separate lots.

G.2.2 For ascertaining the conformity of material to the requirements of this specification, samples shall be tested from each lot separately.

G.2.3 The number of containers to be selected from the lot shall depend on the size of the lot and shall be according to **Tables G.1** and **G.2.**

Table G.1 – Scale of sampling of Fresh Baker's Yeast (FBY)

S/N	Number of packets in the lot	Sample size
1	Up to 50	2
2	51 t0 100	3
3	101 to 150	5
4	151 to 300	7
5	300 and above	10

Table G.2 – Scale of sampling for Dry Baker's Yeast (DBY)

S/N	For containers of less than 100 g		For containers of 100 g to 1 kg		For containers (tins) of 15 kg	
1	Lot size	Sample size	Lot size	Sample size	Lot size	Sample size
2	Up to 100	5	Up to 50	2	Up to 50	2
3	101 to 300	7	51 to 150	3	51 to 150	3
4	301 and above	10	151 to 300	5	151 and above	4
5			301 and above	7		

G.2.3.1 In case of Fresh Baker's Yeast, if packets are packed in cartons, 25 % of the cartons subject to the minimum of two shall be selected and approxiamately equal number of packets shall be selected from each carton. In case the cartons are packed in wooden boxes, at least 255 of the wooden boxes subject to the minimum of two shall be selected and one carton from each such wooden box shall be selected, in order to obtain the requisite sample size according to **Table G.1,** approximately equal number of packets shall be taken from each carton.

G.2.3.2 In case of Dry Baker's Yeast, if containers are packed in wooden boxes, at least 25 % of the wooden boxes subject to the minimum of two shall be selected and approximately equal number of containers selected from each wooden box.

G.2.3.3 The containers from the cartons/wooden boxes and cartons from wooden boxes shall be selected at random from the lot.

G.3 TEST SAMPLES AND REFERENCE AMPLES

G.3.1 Preparation of sample for microbiological examination

In order to draw a representative sample from each container selected, take small portions of the material from a container with an appropriate sampling instrument which is sterile and mix them thoroughly under aseptic conditions. The quantity of material in the sample shall be not less than 100 g. Divide the sample (taking care not to bring in microbiological contamination in the material) into three equal parts; each part shall form reduced sample for that container. A set of such reduced samples, consisting of one reduced sample for each container, shall constitute the test sample. Three sets of these test samples, each being not less than 25 g, shall be transferred to sterile glass containers, sealed air tight and labelled with particulars given in **G.1.4.** They shall be marked, in addition with the words 'For microbiological examination'.

G.3.2 Preparation of sample for other tests

Draw from the portion remaining after taking sample for microbiological examination from each container in the sample, at least 400 g of the container and mix thoroughly so as to form a representative sample for that container. Divide this into three equal portions. Each portion, not being less than 100 g shall be transferred to a clean dry container made of glass or tinplate and labelled with particulars given in **G.1.4.** Three sets of test samples shall be prepared and in addition, be marked with words 'For tests other than microbiological'.

G.3.3 Sample for purchaser o vendor

A set of test samples, consisting of two reduced samples namely one marked 'For microbiological examination' and the other 'For tests other than microbiological', for each container in the sample, shall be sent both to the purchaser and the vendor.

G.3.4 Reference sample

The third set of test samples, consisting of the two reduced samoles, namely one marked ‘For microbiological examination’ and the other ‘For tests other than microbiological’, bearing the seals of the purchaser and the vendor, shall constitute the reference sample to be used in case of a dispute between the purchaser and the vendor.

G.3.5 The samples labelled with words ‘For microbiological examination’ shall be used for determination of the bacterial flora, other than yeast.

G.3.6 The samples labelled with words ‘For tests other than microbiological’, shall be used for all other tests.

G.4 NUMBER OF TESTS AND CRITERIA FOR CONFORMITY

G.4.1 All the characteristics given in **Table 1** shall be tested on individual samples.

G.4.2 Microbiological requirements shall be tested on individual samples labelled with the words ‘For microbiological examination’.

G.4.3 The lot shall be declared as conforming to the requirements of this specification if all the individual test results according to **G.4.1** and **G.4.2** meet the relevant specification requirements.

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