Method 8.19 – Refined sugar: total thermophilic organisms, flat sour spores, anaerobic organisms producing sulphide and anaerobic organisms producing gas

1. Rationale

The method is used for the determination of the total thermophilic organisms, flat sour spores, anaerobic organisms producing sulphide and anaerobic organisms producing gas in canners' sugar.

2. Principle

Diluted aliquots of the sample are mixed with small volumes of cooled molten agar or broth in Petri dishes, bottles or test tubes. Specified selective culture media for thermophilic bacteria are used. The plates, bottles and test tubes are then incubated and the number of colonies which develop or the number of tubes which are positive are counted. Sterile glassware and aseptic technique must be used.

3. Identification

3.1 Thermophilic organisms

The endospores of thermophilic organisms are heat resistant. Vegetative cells of spore-forming bacteria will be killed after a heating period of 5 min at 100°C whilst the growth of the endospores will be stimulated.

3.2 Flat sour spores

The colonies resulting from the growth of flat sour spores are about 2 - 5 mm in diameter, have a characteristic opaque centre and are surrounded by a yellow zone. In the case of those types which are weak acid-producers the yellow zone may not be apparent.

3.3 Anaerobic organisms producing sulphide

Colonies from anaerobic organisms producing sulphide are typically black.

3.4 Anaerobic organisms producing gas

Growth of anaerobic organisms producing gas is evident when a plug inside the test tube is pushed up. The result is expressed as the number of tubes which are positive or negative.

3.5 Pour plates

Pour plates are agar plates which have been inoculated by mixing known amounts of diluted sample with liquid agar medium

4. Apparatus

4.1 Apparatus for dry sterilisation at 170 ± 5°C

This apparatus is used to sterilise glassware.

4.2 Autoclave for sterilisation at 121 ± 2°C

The autoclave is used for sterilisation of water and media.

4.3 Incubator operating at 30 ± 1°C

4.4 Bottles: 25 and 250 cm^3

4.5 Graduated pipettes: 10 cm^3

4.6 Petri dishes: 90 mm ϕ, sterile, disposable, plastic

4.7 Colony counting equipment

4.8 Conical flasks: 500 cm^3

4.9 Test tubes

4.10 Bunsen burner

5. Reagents

5.1 Sterile distilled water

Measure 100 cm^3 distilled water into conical flasks, plug with cotton wool and cover with tin foil. Attach heat sensitive tape to the tin foil covering each flask. The tape will change colour to indicate a successful sterilisation cycle. Autoclave at 121 ± 2°C for 15 minutes.

5.2 Culture media

5.2.1 Dextrose Tryptone Agar (1 000 cm^3)

Dextrose Tryptone Agar is commercially available as Biolab C 69 with the following specifications:

Tryptone	10 g
Dextrose	5 g
Bromocresol purple	0.04 g
Agar	12 g
pH	6.9

5.2.2 Sulphite Agar (1 000 cm^3)

Sulphite Agar is commercially available as Oxoid CM79 with the following specifications:

Tryptone	10 g
Sodium sulphite (anhydrous)	0.5 g
Iron citrate	0.5 g
Agar	12 g
pH	7.1

5.2.3 Cooked Meat Medium (1 000 cm³)

Cooked Meat Medium is commercially available as Biolab C 101 with the following specifications:

Beef heart (defatted)	450 g
Peptone	10 g
Tryptone	10 g
Sodium chloride	5 g
Dextrose	2 g
pH	7.2

5.5 Agar Bacteriological (Agar No. 1) Oxoid L11

5.4 Disinfectant

Commercially available household disinfectant such as "Jik".

6. Procedure

6.1 Sample collection

Samples should be collected in a sterile container and should not be exposed to the atmosphere prior to analysis.

6.2 Preparation of the workplace

Clean and disinfect the working area before commencing analysis. Clearly mark the sterile Petri dishes with the sample name.

6.3 Medium preparation

Following the manufacturer's instructions carefully, rehydrate the culture medium with distilled water. Dispense the medium into bottles and test tubes and sterilise at 121 ± 2°C for 15 minutes. For immediate use the media should be cooled for dispensing into Petri dishes. Alternatively media should be allowed to solidify and be stored in the dark at 0 - 5°C for not longer than 1 month. Media can be remelted.

6.4 Sample preparation

Add 20 g of crystalline sugar aseptically to a 500 cm³ conical flask containing 100 cm³ of sterile distilled water. Shake thoroughly to dissolve the sugar.

Heat the prepared samples quickly to 100°C for exactly 5 minutes. After heating, cool the samples quickly under running water. Include one sterility check in every ten samples by using sterile distilled water instead of sample.

6.5 Inoculation and incubation

6.5.1 Total thermophilic organisms and flat sour spores

Pipette 2 cm³ of the sample solution into each of five sterile petri dishes. Pour approximately 15 cm³ of melted and cooled Dextrose Tryptone Agar into each dish. Carefully mix the inoculum with the medium and allow the mixture to solidify. Invert the dishes and incubate at 55 ± 1°C for 72 hours.

6.5.2 Anaerobic organisms producing sulphide

Add 20 cm^3 of the sugar solution to a bottle containing 50 cm^3 Sulphite Agar, which has been melted and cooled. Mix the contents well and allow to set. Incubate at 55 ± 1°C for 72 hours.

6.5.3 Anaerobic organisms producing gas

Add 3.3 cm^3 of the sugar solution to each of six test tubes containing 10 cm^3 Cooked Meat Medium which has been sterilised and cooled. Seal the medium by pipetting 3.3 cm^3 sterile Agar Bacteriological onto the surface of the medium and allow to set. Incubate at 55 ± 1°C for 72 hours.

Flood the used plastic disposable petri dishes with disinfectant prior to disposal.

7. Expression of Results

7.1 Total thermophilic organisms

Count all the colonies in each plate using the colony counting equipment. Multiply this number by five to calculate the number of CFU per 10 g of sugar.

7.2 Flat sour spores

As the medium contains the indicator bromocresol purple it is also possible to count the number of acid-forming colonies. Count the total number of flat sour colonies on the five plates. Multiply this number by five to obtain the number of flat sour spores per 10 g of sugar.

7.3 Anaerobic organisms producing sulphide

Count the colonies and multiply by 2.5 to get the number of CFU per 10 g of sugar.

7.4 Anaerobic organisms producing gas

Express the result as the number of tubes which are positive or negative.

If any result is more than 300 CFUs, report as > 300 CFU/10 g.

8. Compliance

The total thermophilic organisms should not exceed 100 per 10 g of sugar.

The total number of flat sour spores should not exceed ten per 10 g of sugar.

The total number of anaerobic organisms producing sulphide should not exceed one per 10 g of sugar.

Thermophilic gas-producing anaerobes should not be detected at all in a set of six test tubes.

If the test samples comply with these requirements, the Lot represented by the test samples shall be deemed to comply with the South African Bureau of Standards' specification No. 420 - 1952 for thermophilic organisms in canners' sucrose.

9. References

SABS 420 (1952). Standard specification for canners' sucrose, 13 October, 28 pp.

ICUMSA (1994). Thermophilic spore-forming bacteria. *ICUMSA Methods Book*, GS2/3-49.

SMRI (2004). Determination of the total thermophilic organisms, flat sour spores, anaerobic organisms producing sulphide and anaerobic organisms producing gas in canners' sugar. *SMRI Test Methods*, TM203.