OSMOPHILIC YEAST, MOLD AND BACTERIA (STANDARD PLATE COUNT OR TOTAL PLATE COUNT)

PRINCIPLE

Osmophilic yeast, mold and bacteria are quantitated by the fractional gram pour plate technique. Osmophilic yeast, mold and bacteria are those microorganisms capable of growth in an environment of high osmotic pressures. It is necessary to provide media with reasonable high osmotic pressures for these organisms in order to circumvent osmotic shock and avoid erroneously low quantitative results.

SCOPE

The method is applicable to sugars and syrups of the corn wet milling industry (Note 1).

MEDIA AND REAGENTS

1. Osmophilic Agar: Dextrose, 110 g; Plate Count Agar, 23.5 g, purified water, 1,000 mL. Heat to dissolve, place in flasks and sterilize at 121°C under 15 pounds of pressure for 15 minutes in a steam autoclave. Avoid overheating (Note 2).

2. Osmophilic Dilution Blanks: Dextrose, 400 g; purified water, 1,000 mL. Dispense in dilution blanks in appropriate volumes. Cap and sterilize at 121°C under 15 lbs. pressure for 15 minutes in a steam autoclave. Avoid overheating (Note 2).

3. Filter Sterilized Tartaric Acid Solution, 10%.

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PROCEDURE

Two common fractional gram sample dilution techniques may be used for any given sample. The number of dilutions depends on the individual sample and may be determined by past experience.

1. Factor Five Dilution Series (FFS): Aseptically weigh 20 g of the sample into a sterile 80 mL dilution blank and homogenize. This is the primary 1:5 dilution blank (PDB). Twenty mL of the PDB can be aseptically transferred to another sterile 80 mL dilution blank, and the sample is diluted by a factor of 25.

2. Factor Ten Dilution Series (FTS): The same as FFS only 10 g of sample and 90 mL sterile dilution blanks are used. The sample is diluted by a factor of 10, 100, 1000, etc.

   Aseptically dilute the sample by either FFS or FTS. Pipet aseptically 1 mL of each dilution to appropriately marked duplicate Petri dishes. Pour 15-20 mL of the media of choice which has been cooled to 45°C into each dish. Swirl plates and allow to solidify.

A. Osmophilic Bacteria:

   Invert plates and incubate at 35-37°C for 48 ± 3 hours (2 days). Count the number of colonies on those plates showing 25-250 colonies. Average the count of the duplicate plates, multiply by the dilution factor and record as the number of bacteria per gram. If the lowest dilution shows less than 25 colonies, then these colonies must be counted and reported.

B. Yeast and Mold:

   Invert the plates and incubate at 25-30°C. Count the plates after 72 hours (3 days), but if the colonies are too small, extend the incubation time to 96-120 hours (4-5 days). Count the number of yeast and mold colonies on the plates, average the count of duplicates, multiply by the dilution factor and

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record as the number of yeast and/or mold per gram or mL. Yeast and mold are recorded separately.

CALCULATION

Number of bacteria per gram = Average number of bacteria x Dilution Factor

Number of yeast/mold per gram = Average number of yeast/mold x Dilution Factor

NOTES AND PRECAUTIONS

1. The membrane filter technique (CRA Microbiological Method III-B) may be used for sugars and syrups if low counts are anticipated.

2. Avoid overheating osmophilic media, as overheating will hydrolyze the agar and form excessive amounts of hydroxymethylfurfural (HMF).

REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.