

## OSMOPHILIC YEAST, MOLD AND BACTERIA (MEMBRANE FILTER METHOD)

### PRINCIPLE

Osmophilic yeast, mold and bacteria can be quantitated by a membrane filtration technique. Selection and quantitation of osmophilic yeast and mold is achieved through use of media of relatively high osmotic pressure. In addition, dilution blanks of high osmotic pressure are used to minimize osmotic shock to microbial cells during the filtration process. Use of the membrane filtration technique allows accurate quantitation of osmophilic yeast, mold and bacteria when low counts are anticipated.

### SCOPE

The method is applicable to the sugars and syrups of the corn wet milling industry.

### SPECIAL APPARATUS

1. Smooth-tipped, stainless steel forceps
2. 47 mm grid marked, white sterile 0.45  $\mu\text{m}$  membranes and 47 mm absorbent pads (Millipore HAWG 047S0, HAWG S2 or equivalent)
3. Autoclavable 47 mm filtration systems with holder base, funnel assembly and receiver flask (Millipore or equivalent)
4. Vacuum pump capable of 22-27 inches of vacuum
5. Sterile 47 mm plastic petri dishes

### 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 at 15 pounds of pressure for 15 minutes in a steam autoclave (Note 1). For quantitation of osmophilic yeast and mold, aseptically add 2 mL of sterile

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10% tartaric acid solution to each 100 mL of sterile and cooled (45°C) medium prior to use. For quantitation of osmophilic bacteria, tartaric acid is not added to the osmophilic agar.

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 1).
3. Filter Sterilized Tartaric Acid, 10%
4. Ethanol
5. Sodium Hydroxide Solution, 1*N*
6. 47 mm petri dishes plus medium: Prepare 47 mm petri dishes by pouring approximately 2-3 mL of sterile and properly prepared media into each of the dishes. Allow the media to solidify.

**PROCEDURE**

1. Aseptically weigh 25 g of the sample into a sterile 50 mL osmophilic diluent blank and homogenize.
2. Assemble a sterile 47 mm filtration system with a 47 mm grid marked, white 0.45 µm pore size membrane (Note 2). Connect the receiver flask to the vacuum pump using the vacuum hose.
3. Aseptically pour all of the homogenous sample solution into the filter funnel and then cover the funnel top opening. Apply vacuum (22-27 psi) and filter the sample solution through the membrane filter. If desired, sterile osmophilic diluent can be used for rinsing of the funnel.
4. Using sterile forceps transfer the membrane to a petri dish containing solidified, sterile medium. Roll the filter onto the medium in such a

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manner to avoid air bubbles forming between the membrane and the medium surface.

5. Incubate.
  - A. Osmophilic yeast and mold: incubate the petri dish containing the membrane at 25-30°C for a minimum of 72 hours or a maximum of 120 hours (3-5 days). Using a lighted magnifier count separately all the yeast and mold colonies. The membrane grid can be used as an aid when scanning the filter (low power magnification). The total counts of osmophilic yeast and mold colonies represent the number of each type organisms per 25 g of sample (as is).
  - B. Osmophilic bacteria: incubate the petri dish containing the membrane at 35-37°C for a minimum of 48 hours and a maximum of 72 hours (3 days). Determine the number of colonies (osmophilic bacteria) per 25 g of sample (as is) as described above for osmophilic yeast and mold.

**CALCULATION**

The number of osmophilic yeast, mold or bacteria can be reported either as is or on a dry solids basis:

$$\text{As is basis (per 10g)} = \frac{\text{Osmophilic yeast, mold or bacteria/25g sample (as is)}}{2.5}$$

**NOTES AND PRECAUTIONS**

1. Avoid overheating osmophilic media as overheating will hydrolyze the agar and form excessive amounts of hydroxymethylfurfural (HMF).
2. The membrane can only be handled with sterile forceps. Sterilize the forceps by keeping the forcep blades in 1/2" of ethanol and then igniting the alcohol to burn itself out just prior to handling the membrane.

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**REFERENCE**

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