3. ENUMERATION OF YEAST AND MOULD

SCOPE:

To enumerate yeast and mould in raw materials, Coconut milk and Desiccated Coconut products by counting the colonies growing in a solid medium after aerobic incubation at 25 ± 1ºC.

PRINCIPLE:

Yeast and mould are defined as fungal cells capable of forming colonies under specified conditions. Sample dilutions are plated on chloramphenicol yeast glucose agar (CYGA) which contains antibiotic chloramphenicol to suppress the growth of bacteria. Plates are incubated at 25 ± 1ºC for five days to enumerate yeast and mould.

DILUENT, CULTURE MEDIA AND REAGENTS:

- Peptone salt water
- Chloramphenicol yeast glucose agar (CYGA)

APPARATUS, INSTRUMENTS AND GLASSWARES:

- Laminar airflow chamber
- Autoclave
- Hot air oven
- Sterile petri plates
- Colony counter
- Weighing balance
- Dilution bottles
- Water bath
- Test tubes
- Sterile spatula
- Micropipette
- Sterile tips

PREPARATION OF TEST SAMPLE:

1. Desiccated coconut

All the samples are aseptically mixed in order to make the sample uniform.

2. Coconut milk

All the samples are aseptically mixed in order to make the sample uniform

PROCEDURE:

- Aseptically transfer 10 ml/10 gm of sample into 90 ml of diluent in dilution bottles. Mix the sample by shaking 25 times in 7 seconds with a 1 foot (30cm) movement. This will give 10^{-1} dilution.
- Aseptically transfer 1 ml sample from 10^{-1} dilution into a tube containing 9 ml dilution blank and mix well. This result in 10^{-2} dilution. Accordingly make serial dilutions depending on the expected count of the product tested.
- Pipette 1ml of each dilution into a separate, duplicate and appropriately marked petri plates.(For liquid products (eg. Milk) direct sample can also be plated).
- Use a new sterile pipette for each decimal dilution.
- Pour about 15 ml of CYGA at 45ºC ± 0.5ºC into each plate within 15 minutes of initial dilution preparation.
- Carefully mix the inoculum with the medium by rotating the petri plates and allow the mixture to solidify on a cool horizontal place.
- After solidification invert the plates and incubate at 25 ± 1ºC for 5 days.

COUNTING OF COLONIES:

Count the colonies on each plate after 3, 4 and 5 days of incubation. After 5 days, retain those plates containing fewer than 150 colonies. If plates are overgrown after 5 days of incubation, take the counts obtained after 3rd or 4th days for calculation. In this case, the test report specifies incubation period. (eg. 3 days or 4 days).

EXPRESSION OF RESULT:

Use counts from plates containing fewer than 150 colonies.

The number of yeast and mould per gram or per milliliter is equal to:

$$\frac{\sum c}{(n_1 + 0.1n_2)\, d}$$

Where

Σc = is the sum of the colonies counted on all the plates;
n_1 = is the number of plates retained in the first dilution;
n_2 = is the number of plates retained in the Second dilution;
d = is the dilution factor corresponding to the first dilution.

Round the result calculated to two significant figures.

Report result as number of CFU /gm or ml of the sample.

If there were no colonies on plates from the initial suspension, if the initial product was solid, the number of yeasts and moulds per gram of product should be reported as fewer than 10 (<10) cfu/gm.

If there were no colonies on plates from the initial suspension, if the initial product was liquid, the number of yeasts and moulds per milliliter of product should be reported as less than 1(<1) cfu/ml.

REFERENCE:

IS 5403: 1999.ISO 6611:2004