

## Enumeration of total fungal load in cured fish and shellfish

Fungi comprise a large group of eukaryotic, non-photosynthetic, saprophytic organisms widely distributed in nature. These are responsible for the spoilage and quality deterioration of fish and fishery products of low water activity such as dried fish. Fungi are characterized by the presence of vegetative mycelia as well as variety of spores with variety of coloration which aid in grouping them in to different groups. Suitable laboratory mediums with low pH or antibiotics which prevent the growth of bacteria are employed for cultivating fungi. The common spread plate technique is useful in enumerating fungal load in test sample. However, plate count does not give correct information as the fungal hyphae which get fragmented during sample preparation also develop as colonies along with spores.

### Materials required

**1. Diluent** : Phosphate buffer or Physiological saline (0.85%)

**2. Medium** : Czapek-Dox Agar , Potato dextrose agar(PCA), Sabouraud agar

Any one of the above medium can be used.

### Procedure

About 25 g of sample is weighed aseptically and homogenised with 225 ml physiological saline in a homogeniser / blender. Appropriate serial decimal dilutions (  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  ) depending on the type of sample are prepared with 9 ml physiological saline. 0.1 ml of inoculum from each of the serial dilution is poured onto prepared and dried agar plates and spread using sterile glass spreaders. The plates are incubated at inverted position at room temperature for 4- 5 days. All the colonies developed on the agar plates are then counted and counts per gram of sample calculated. It is advisable to choose dilutions which give colonies between 30 – 300 ranges.

### Calculation

Count (CFU) / g of sample = (Number of colonies x Dilution x 10)/Weight of sample

### Media composition

#### *Phosphate Buffer Solution*

##### *Stock solution*

Potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ )	-	34.0 g
Distilled water	-	1000.0 ml

##### *Working solution*

Dilute 1.25 ml of stock buffer solution to 1000 ml with distilled water. Adjust the pH to 7.2 before use. Sterilize at  $121^\circ\text{C}$  for 15 minutes.

##### *Czapek-Dox Agar*

Sodium nitrate	2.0 g
Potassium chloride	0.5 g
Magnesium sulphate, hydrated ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	0.5 g
Dipotassium hydrogen phosphate	1.0 g
Ferrous sulphate, hydrated ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )	0.01 g
Sucrose	30.0 g
Agar	15.0 g
Distilled water	1000 ml

Dissolve ingredients in water by steaming, dispense as required in conical flasks and sterilize at  $115^\circ\text{C}$

for 15 minutes.

***Sabouraud agar (pH: 5.6)***

Peptone	10 g
Dextrose	40 g
Agar	15 g
Distilled water	1000 ml

Dissolve ingredients in water by steaming, adjust pH to 5.6, dispense as required in conical flasks and sterilize at 121<sup>0</sup>C for 15 minutes.