AEROBIC PLATE COUNT

LIM P. Y.

INTRODUCTION

The aerobic plate count provides an estimate of the number of viable micro-organisms in food according to the medium used and the time and temperature of incubation. The spread plate method described below is based on the assumption that each viable cell will form a colony, thus it is important that:-

— the sample is adequately dispersed

— the cells do not lose their viability

— the cells do not multiply during the preparation of the dilutions.

The material under investigation is diluted in known volumes of sterile diluent to provide a set of serial dilutions of the microbial population so that an aliquot at some step in the series provides 30 to 300 colonies when plated on a nutrient medium. (It is this count that will give the most accurate colony count.)

I   CULTURE MEDIA*

Plate count agar (PCA) or Standard Methods agar

Butterfield's buffered phosphate diluent.

* Refer to Appendix B for methods of media preparation.

II   APPARATUS

'Waring' blender & flasks	Autoclave
Pipettes	Incubator
Scissors & forceps	Water-bath
Alcohol lamps	Weighing balance
Alcohol (70% v/v) swabs	Laminar flow chamber
Bent glass spreader	

III   SAMPLING PROCEDURE

Randomly pick 150-200 g of sample. Aseptically slice each piece of the sample in half and keep the half-cut portions in a sterile polyethylene bag or container. Store in refrigerator (5°C) to maintain its integrity.

IV   SAMPLE PREPARATION

1.   Weigh about 50 g of the above sample and put them into a 'Waring' blender flask. Add in 450 ml of sterile Butterfield's buffered phosphate diluent. Blend for 1 min at low speed.

2.   Transfer 5 ml of this suspension into 45 ml of phosphate diluent to give a dilution of 10^{-1}. Prepare further dilutions by mixing 1 ml of the well mixed diluted sample solution with 9 ml of phosphate diluent.

V PROCEDURES

1. Select the appropriate dilutions and for every dilution, inoculate 0.1 ml aliquots to each of two PCA plates.

2. Spread the inoculum gently and evenly over the surfaces of the agar plates with a sterile bent glass spreader.

3. Allow the plates to stand until the inoculum has been absorbed completely, which should be within 15 mins after the spreading.

4. Invert the plates and incubate at 35°C for 48 hrs or at any suitable temperature and period.

5. Count those plates which have between 30-300 colonies.

6. The Aerobic plate count for the sample is calculated as below:-

    Average number of colonies on the plate × Dilution factor × 10

    Calculate to per gram of the sample. (See below).

VI CALCULATION OF AEROBIC PLATE COUNT (Spread Plate Method)

$$APC = \frac{450 + W}{W} \times \frac{1}{d} \times 10 \times \frac{En + En'}{p + (0.1)q} \quad \text{organisms/g}$$

where   d   :   lowest dilution

En   :   total count of 2 plates at lowest dilution

En' :   total count of 2 plates at highest dilution

p   :   number of plates at lowest dilution

q   :   number of plates at highest dilution

W   :   weight of sample

VII BACTERIOLOGICAL LIMITS OF APC FOR FISH/FISHERY PRODUCTS (COOKED & RAW)

Total plate count at 35°C for 48 hrs for

cooked products : 1×10^6 orgs/g

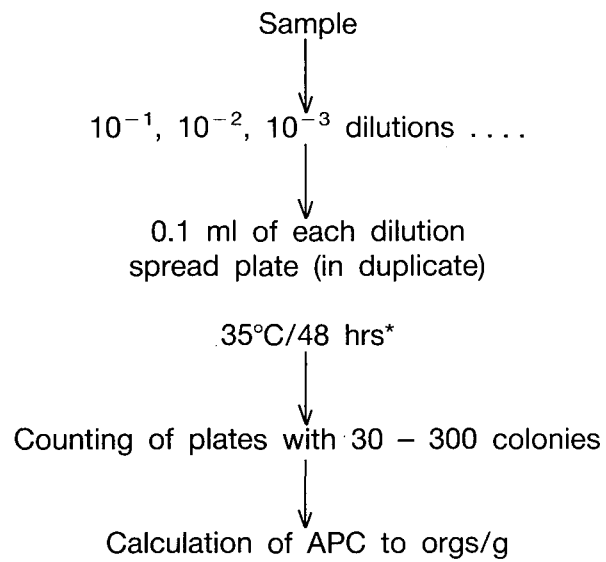raw products   : 2.5×10^6 orgs/g

REFERENCES

A. Hazzzard. (1985) ASEAN Training Course in Fish Quality Control. Training Course organised by HAWKAID, Hawkesbury Agricultural College Research and Development Co. Ltd. Chapter: Fish quality control microbiology. Section 4:63-65

Veterinary Public Health Laboratory, Primary Production Department unpublished manual.

A flow diagram of the procedure for Aerobic Plate Count (APC) is included as the following figure.

FLOW DIAGRAM OF THE PROCEDURE FOR AEROBIC PLATE COUNT (APC)

Sample

$\downarrow$

10^{-1}, 10^{-2}, 10^{-3} dilutions . . . .

$\downarrow$

0.1 ml of each dilution
spread plate (in duplicate)

35°C/48 hrs*

$\downarrow$

Counting of plates with 30 − 300 colonies

$\downarrow$

Calculation of APC to orgs/g

* or any suitable temperature and period of incubation.