EXPERIMENT 9 DETERMINATION OF THE EFFICIENCY OF PASTEURIZATION

Structure

9.1 INTRODUCTION

One of the most important aspects of pasteurization is to make the milk safe for human consumption by destroying pathogenic organisms present in it. Heating time temperature combination for pasteurization has been determined on consideration of inactivation of constitutive alkaline phosphates enzyme, which is virtually destroyed under conditions of efficient pasteurization.

9.2 OBJECTIVES

- determine whether the pasteurization of milk ensures destruction of all the pathogens.
- assess the extent of post pasteurization contamination.
- know inadequate heating of milk.

9.3 EXPERIMENT

i. Principle

Phosphatase of raw milk is destroyed at the temperature –time combination of heating necessary for efficient pasteurization. To test whether the heat treatment by holding or HTST method is properly carried out, the treated milk is subjected to the phosphatase test which helps to indicate the presence or absence of phosphatase enzyme. Phosphatase present in milk is destroyed by just about the same heat treatment necessary for the destruction of *Mycobacterium tuberculosis*, the most heat resistant index pathogen likely to be present in milk. When heat treatment is less than that specified in the method, some of the phosphatase remains active and will liberate phenol (blue) from disodium phenylphosphate or paranitrophenol (yellow) from r-nitrophenyl disodium orthophosphate under the alkaline conditions of test. The colour is a measure of the phosphatase content of the milk sample. Therefore, if phosphatase is present, it follows that the milk has been inadequately heated, or has been contaminated after the heating process by raw milk.

ii. Requirements

1. Equipments and apparatus:

i) The all-purpose Lovibond Comparator complete with stand for work in reflected light.

ii) Standard discs, giving 0,6,10,18,42,or 0,6,10,14,18,24,42 readings

iii) Fused glass cells-25 mm : 2Nos.

iv) A water bath at 37.5°C +/- 0.5 °C.

v) Pipettes: 5ml, 1ml straight sided, NPL grade B specification.

vi) Test tubes: 15 ml 1.9 cm with ring at 10 ml fitted with rubber stoppers.

vii) Graduated flask : 1000ml- one

viii) Measuring cylinder : 100ml- one

2. Chemicals and reagents

i) Buffer solution: 3.5 g anhydrous Na2CO3 (Sodium Carbonate) and 1.5 g of NaHCO3 (sodium bicarbonate) dissolved in water and made upto 1 litre.

ii) Substrate: r-nitrophenyl disodium orthophosphate not less than 95% pure.

iii) Buffer substrate solution: Transfer 0.15 g of the substrate into a 100 ml measuring cylinder or stoppered graduated flask and make up to mark with the buffer solution. The solution should not be stored for long periods but may normally be kept protected from light in a refrigerator for upto one week. The solution is practically colourless; when viewed through a 25 mm cell in the All-purpose comparator, it should give a reading of less than “10” on the disc.

iii. Procedure

i) Fill 5 or 10 ml of the buffer substrate solution into test tubes marked at 5 or 10 ml and heat to 37-38 deg.C in a water bath.

ii) Add 1 or 2ml (depending on 5 or 10 ml of buffer substrate are used) of the milk to be tested, close the tubes with rubber stoppers and invert to mix.

iii) Prepare in the same way a blank from a boiled milk of the same type as that under test.

iv) Incubate all the tubes at 37-38°C for 2 hours.

v) Read the yellow colour after 30 minutes, return to the water bath and take a second reading after incubation for a further 90 minutes. The yellow colour is read in a Lovibnd All Purpose Comparator on a resazurin stand, fitted with the disc calibrated in microgram of: r-nitrophenol.

vi) The blank is placed on the left of the stand and the sample on the right.

vii) Reading are taken by looking down on to the two appertures with the comparator facing a good source of day light; the disc is revolved until the sample is matched; readings falling between two standards are recorded to the nearest reading.

viii) After a further 20 minutes of incubation, remove the test tubes from the water bath and mix them well by gradual inversion and read the colour as before.

iv. Observations

Record the disc readings:

Time of incubation (min)	Disc readings
0	
30	
60	
90	
120	

v. Results

Interpretation of result is done as follows:

i) Disc reading after 30 min incubation	Interpretation
0 or trace	Properly pasteurized.
6	Doubtful
10 or over	Under pasteurized

ii) Disc reading after 2 hour incubation	Interpretation
0 to 10	Properly pasteurized
Over 10 to 18	Slightly under pasteurized
Over 18 to 42	Under pasteurized
Over 42	Not pasteurized

9.4 PRECAUTIONS

i) The 30 min. test will reveal only serious fault in pasteurization but 2 hour test enables minor errors to be detected.

ii) Positive phosphatase test in pasteurized milk may be due to:
   a) Inadequate holding period
   b) A temperature indicated by recorder is faulty.
   c) Contamination with raw milk.