Practical Exercise 10

Determination of efficiency of pasteurization

Objective

Determination of efficiency of pasteurization

Requirements

A. Buffer solution: 1.5 g of sodium bicarbonate and 3.5 g of anhydrous sodium carbonate are dissolved in water to make it one litre. Store in a refrigerator and discard after 1 month.

B. Disodium p- nitrophenylphosphate. The solid substrate must be kept in the refrigerator.

C. Buffer-substrate solution - Weigh accurately 0.15 g of substrate (disodium p-nitrophenyl phosphate) into a 100 ml measuring cylinder and make it up to 100 ml with buffer solution. The solution should be stored in refrigerator and protected from light. The solution should give a reading of less than the standard marked 10 on comparator disc APTW or APTW 7 when viewed through a 25 mm cell (distilled water is used as a blank). The solution must be discarded after one week.

D. A Lovibond Comparator with stand for work in reflected light.

E. A lovibomd comparator disc APTW or APTW 7.

F. Two Fused glass cells of 25 mm depth.

G. A water bath or incubator capable of being maintained at 37.5 ± 0.5°C.

H. 1 ml pipette and 5 ml pipette.

I. 1 litre graduated flask.

J. 100 ml measuring cylinder.

K. Test tubes, nominal size 150/16 mm with rubber stoppers.

Introduction

Alkaline phosphatase enzyme is naturally present its milk. The enzyme activity is destroyed at pasteurization temperature and has been adopted as an index of the efficiency of pasteurization. Since milk is a proven vector for a number of pathogenic bacteria, including Salmonella, Campylobactor and Listeria, the test is of very great significance to the dairy industry as a means of policing the thoroughness of heat treatments or the addition of raw milk to heated or unheated products In the following method, a solution of disodium p-nitrophenyl phosphate in a buffer of pH 10.2 is used as substrate. This compound, colourless in solution, is hydrolyzed by alkaline phosphatase of milk to liberate p-nitrophenol, which under alkaline condition gives an intense yellow colouration to the solution. The liberated p-nitrophenol is measured by direct comparison with standard colour discs in a Lovibond comparator. The test does not apply to sour milk and milk preserved with chemical preservatives.

Procedure

In a test tube pipette, 5 ml of buffer substrate solution, place stopper and bring the temperature to 37°C. Add 1 ml of test milk to it, shake and replace stopper, incubate at 37°C for 2 hrs. Incubate one blank prepared from boiled milk of the same type as that undergoing the test with each series of sample. Remove the tubes after 2 h and the content should be well mixed. Place the boiled milk blank on left hand side of the comparator stand and test sample on the right. Take reading in reflected light by revolving the disc until the test sample is matched. Record readings falling between two standards by affixing a plus or minus sign to the figure for the nearest standard.

The test is considered satisfactory if it gives a reading of 10 µg or less of p-nitrophenyl per ml of milk. Properly pasteurized milk gives no discernible colour.

Reference

FSSAI.2012. Manual of methods of analysis of foods. Milk and milk products. Ministry of Health and Family Welfare, GOI, New Delhi

REVIEW QUESTIONS

1. What is the importance of alkaline Phosphatise test?
2. Describe procedure for determination of pasteurization efficiency.
3. List reagents for pasteurization efficiency test.