

Possibility of reactions in Ice-cream

Since ice cream is a mixture made from cream, milk, condensed milk or milk powder, sugar, gelatin, fruits, coloring and flavoring, it does not necessarily follow that the results of the phosphatase test on ice cream would be comparable to those obtained on milk. The contribution of phenolic compounds from fruits, flavours and color from colouring compounds etc to ice cream leads to false positive results.

In view of above, the note given at the end of clause H 3.1 of Appendix H of IS 2802: 1964 and the principle mentioned below, the following is proposed:

Principle :

- When milk containing alkaline phosphatase enzyme is mixed and incubated with its substrate - disodium phenyl phosphate, the enzyme in raw milk cleaves a phosphate group from disodium phenyl phosphate and liberates phenol.
- The liberated phenol then reacts with a color producing compound -2,6 dichloro - quinone - chloramide (CQC) to give a blue color.
- Intensity of blue colour depends upon the enzyme activity present in the milk
- If more the enzyme, more phenol will be liberated giving a deeper blue color.
- The intensity of the blue can be observed visually or with a spectrophotometer.
- The test can be qualitative or quantitative.
- The results of quantitative test are expressed as micrograms of phenol per ml of milk.
- A amount of more than 1 microgram is indicative of improper pasteurization.
- Highly sensitive test & it detects contamination of pasteurized milk just by 0.1 % of raw milk.

The diagram illustrates the chemical reaction process. It starts with Disodium Phenyl Phosphate reacting with Alkaline Phosphatase to produce Phenol and Phosphate. Phenol then reacts with 2,6-dichloro-quinone-chloramide (CQC) to form Indophenol, which is responsible for the blue color.

The schematic diagram shows a test tube containing 1 ml Milk Sample and 5 ml Disodium Phenyl Phosphate. The tube is incubated for 2 hours in the presence of CQC. The result is a blue color in the presence of phosphatase and no color change in its absence.

1. The IS 1479(Part 2)- 1961 referred in Appendix H of IS 2802: 1964 is not suitable for testing phosphatase enzyme as detection of yellow colour end point in ice cream is not possible. Therefore, referred IS in Appendix H of IS 2802: 1964 is to be replaced with IS 8479 (Part 2) : 2020 Milk — Determination of Alkaline Phosphatase Part 2 Reference Method (First Revision).
2. The following may be suitably added at end of Appendix H of IS 2802: 1964
 - No blue colour development in sample test tube indicates that ice cream is pasteurized and phosphatase test is negative and vice-versa.
 - Development of blue colour in the blank test tube (clause 8.2.2 of IS 8479 (Part 2) : 2020) indicates interference of Phenol contributed by flavouring ingredients and/or fruits added in the ice cream. In such case, If colour developed in blank test tube is equal in intensity to colour developed in sample test tube, then this indicates that ice cream is pasteurized and phosphatase test is negative. If intensity of blue colour develop in sample test tube is more than blank test tube, then this indicates that ice cream is under pasteurized and phosphatase test is positive.
 - In place of Dibromoquinonechloroimide (BQC), the Dichloroquinonechloroimide (CQC) may also be used.