Australian Standard®

Methods of chemical and physical testing for the dairying industry

Method 1.10: General methods and principles—Determination of phosphatase activity

PREFACE

This Standard was prepared by the Standards Australia Committee, FT-024, Food Products and Subcommittee FT-024-05, Dairy Products to supersede AS 2300.1.10—1988. Phosphatase activity is of microbiological significance in that it is an indication of the efficiency of the pasteurization process.

After a periodic review, the Committee recommended a new edition. This edition confirms the method without technical changes but updates the referenced documents and reflects the current editorial style and includes a clause on uncertainty of measurement.

AS 2300 comprises a series of methods and related Standards for chemical and physical testing of milk and dairy products, including the preparation of samples for testing.

Standards in the AS 2300 series are divided into categories according to type of product to be tested, as follows:

AS	
2300.1	General methods and principles
2300.2	Liquid milks
2300.4	Dried milk and dried milk products
2300.5	Condensed milk
2300.6	Cheese
2300.7	Butter
2300.8	Anhydrous milk fat
2300.9	Analysis of ice-cream and frozen milk products
2300.10	Caseins, caseinates and coprecipitates
2300.11	Cultured milk products

METHOD

1 SCOPE

This Standard sets out chemical tests for determining the amount of alkaline phosphatase enzyme (phosphomonoesterase) activity present in a sample of milk or milk product.

NOTE: Phosphatase activity is used for assessing the efficiency of pasteurization.

2 APPLICATION

The method is applicable to whole milk, skim milk, fortified low fat milk, flavoured milk, cream, dried milk products and cheese. It may not be suitable for testing some coloured products and those which contain relatively high proportions of other materials, e.g. coloured cheese powders, cocoa or chocolate crumb. It is not suitable for testing goat's milk or goat's milk products, nor is it applicable to products subjected to ultra-heat-treatment (UHT).

3 REFERENCED DOCUMENT

The following documents are referred to in this Standard.

AS
1166 Milk and milk products—Guidance in sampling

AS/NZS
2243 Safety in laboratories
2243.2 Part 2: Details regarding laboratory safety

4 PRINCIPLE

The tests are based on the work of Aschaffenburg and Mullen.

The liquid or reconstituted sample is diluted with a buffer substrate at pH 10.2 and incubated at 37.5°C for 2 h. Any residual alkaline phosphatase present in the sample will, under these conditions, liberate *p*-nitrophenol from the substrate (*p*-nitrophenyl phosphate). The liberated *p*-nitrophenol is measured by one of a number of alternative methods, as follows:

(a) By comparator, for rapid, routine purposes.

(b) Colorimetrically, for reference purposes.

(c) Spectrophotometrically, for samples in which masking of the colour precludes visual assessment, e.g. flavoured milks and some cheeses.

5 REACTION

$$p\text{-nitrophenyl phosphate} + \text{alkaline phosphatase} \xrightarrow[37.5\,^{\circ}\text{C}]{pH\ 10.2} p\text{-nitrophenol}$$

WARNING: THE USE OF THIS STANDARD MAY INVOLVE THE USE OF HAZARDOUS MATERIALS, OPERATIONS AND EQUIPMENT. THIS STANDARD DOES NOT PURPORT TO ADDRESS ALL THE SAFETY RISKS ASSOCIATED WITH ITS USE. IT IS THE RESPONSIBILIYT OF THE USER OF THIS STANDARD TO ESTABLISH APPROPRIATE SAFETY AND HEALTHY PRACTICES AND DETERMINE THE APPLICABILITY OF LOCAL REGULATORY LIMITATIONS PRIOR TO USE. SEE AS/NZS 2243.2 FOR MORE DETAILS REGARDING LABORATORY SAFETY.

6 REAGENTS

6.1 General requirements

Use only reagents of recognized analytical reagent grade, and freshly distilled water or water of equivalent purity. The storage conditions stipulated should be observed to ensure that the quality of the reagents does not deteriorate.

6.2 Reagent solutions

The following reagents are required:

(a) *Buffer solution pH 10.2*

Dissolve 3.5 g of anhydrous sodium carbonate (Na_2CO_3) and 1.5 g of sodium bicarbonate ($NaHCO_3$) in water in a 1000 mL volumetric flask and make up to volume.

(b) *Buffer solution, double strength (for cheese)*

Dissolve 7.0 g of anhydrous sodium carbonate and 3.0 g of sodium bicarbonate in water in a 1000 mL flask and make up to volume.

(c) *Buffer substrate solution*

Transfer 0.15 g of disodium p-nitrophenyl phosphate hexahydrate ($NO_2C_6H_4OPO_3Na_2.6H_2O$) to a 100 mL volumetric flask, dissolve and make up to volume with buffer solution. Store the solution at approximately 4°C and protect from light. Discard the solution if its colour exceeds that of a mixture of 1 mL of the 6 µg/mL standard solution of p-nitrophenol (see Item (g)) with 5 mL of buffer solution.

Alternatively, discard if the colour exceeds disc No.6 when examined against buffer solution in the comparator.

NOTE: For maximum life keep the reagent (disodium *p*-nitrophenyl phosphate) in the dark at a temperature not exceeding 4°C.

(d) *Buffer substrate solution in double strength buffer (for cheese)*

Prepare as described in Clause 6.2(c) above, using the double strength buffer (see Clause 6.2(b)).

(e) *p-nitrophenol stock solution*

Dissolve 250 ±2 mg of p-nitrophenol ($NO_2C_6H_4OH$) in water in a 100 mL volumetric flask, and make up to volume. Store away from light.

NOTE: When stoppered and stored under refrigeration, this solution may be kept for one month.

(f) *Diluted p-nitrophenol stock solution (50 µg/mL)*

Dilute 2.0 mL of the p-nitrophenol stock solution to 100 mL with water in a 100 mL volumetric flask. Store away from light. 1 mL of this solution contains 50 µg p-nitrophenol.

NOTE: When stoppered and stored under refrigeration, this solution may be kept for one week.

(g) *Standard solutions of p-nitrophenol*

Prepare appropriate standards by transferring the required quantities of the 50 µg/mL standard into 50 mL volumetric flasks and making up to volume with water. The number of millilitres of the 50 µg/mL stock solution is numerically equal to the required number of micrograms per millilitre of p-nitrophenol in the standard solution when made up to 50 mL, e.g. for a 1 µg/mL standard, dilute 1 mL of the 50 µg/mL solution to 50 mL.

NOTE: The standard solutions should be stored away from light and made up daily.

(h) Protein precipitant (for cheese)

Dissolve 25 g of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) in water and dilute to 100 mL.

(i) Sodium hydroxide 0.5 mol/L (for cheese)

Dissolve 20 g of sodium hydroxide in water and dilute to 1 L.

(j) Chloroform (for flavoured milks).