Australian Standard®

Methods of chemical and physical testing for the dairying industry

Method 1.9: General methods and principles—Determination of sucrose and glucose—Enzymatic method

PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Subcommittee FT-024-05, Dairy Products, to supersede AS 2300.1.9—1987.

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

FOREWORD

This method is based on enzymatic procedures but it should be recognized that other procedures using more advanced instrumentation are available, for example, HPLC.

METHOD

1 SCOPE

This Standard sets out an enzymatic method for the determination of sucrose and glucose in liquid milks, flavoured milks, yogurts, ice cream and other frozen milk products.

2 REFERENCED DOCUMENTS

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: Chemical aspects

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 RESPONSIBILITY 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.

3 PRINCIPLE

The method is based on a coupled enzyme system in which sucrose is hydrolysed to glucose and fructose. The glucose is subsequently oxidized, resulting in the quantitative formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The absorbance of the NADPH is proportional to the glucose present and, by measurement of absorbance of NADPH before and after hydrolysis of sucrose, the amounts of sucrose and glucose in the sample are determined.

4 REACTIONS

The reactions that occur are as follows:

(a) $\text{Sucrose} + H_2O \xrightarrow{\text{fructosidase}} \beta\ \text{glucose} + \text{fructose}.$

(b) $\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}.$

(c) $\text{Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{G-6-P-DH}} \text{6-phosphogluconate} + \text{NADPH} + H^+.$

Reactions (b) and (c) are also carried out in the absence of reaction (a) to determine amount of glucose originally present.

5 REAGENTS

5.1 General requirement

Use only reagents of recognized analytical reagent grade. To ensure adequate shelf life of prepared solutions, only water of double distilled or equivalent purity should be used.

NOTE: Reagents that are commercially available in kit form should be used in accordance with the instructions supplied.

5.2 Reagents

The following reagents are required:

(a) *Sucrose, standard solution* Dissolve 0.5 g to 0.7 g of dried sucrose, weighed to the nearest 0.001 g, in water in a 1000 mL volumetric flask and make up to the mark. Prepare on the day of use.

NOTE: Dry the sucrose at 105°C for 3 h, or alternatively in a vacuum oven at 70°C ±5°C overnight.

(b) *Carrez I solution* Dissolve 3.6 g potassium hexacyanoferrate-II trihydrate, $K_4(Fe(CN)_6).3H_2O$ in about 70 mL water and dilute to 100 mL with water.

(c) *Carrez II solution* Dissolve 7.2 g zinc sulphate heptahydrate, $ZnSO_4.7H_2O$ in about 70 mL water and dilute to 100 mL with water.

(d) *Citrate buffer* 0.32 mol/L, pH 4.6. Dissolve 6.9 g citric acid monohydrate ($C_6H_8O_7.H_2O$) and 9.1 g trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) in about 150 mL water, adjust to pH 4.6 with 2 mol/L NaOH and dilute to 200 mL with water.

NOTE: This solution is stable for 1 year at 4°C.

(e) β-*fructosidase*, 5 mg/mL. Dissolve 10 mg β-fructosidase in 2.0 mL of citrate buffer (see Clause 5.2(d)).

NOTE: This solution is stable for 4 weeks at 4°C or 2 months at –20°C.

(f) *Triethanolamine buffer*, containing 0.75 mol/L triethanolamine and 10 mmol/L magnesium sulphate, pH 7.6.

Dissolve 14.0 g triethanolamine hydrochloride and 0.25 g magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) in 80 mL water, adjust to pH 7.6 with 5 mol/L NaOH and dilute to 100 mL with water.

NOTE: This solution is stable for 4 weeks at 4°C or 2 months at –20°C.

(g) Nicotinamide adenine dinucleotide phosphate (NADP+) solution, approximately 12 mmol/L aqueous solution. Dissolve 50 mg NADP-$Na_2.3H_2O$ in 5.0 mL of water.

NOTE: This solution is stable for 4 weeks at 4°C or 2 months at –20°C.

(h) *Adenosine triphosphate (ATP) solution*, approximately 81 mmol/L aqueous. Dissolve 250 mg of the trihydrated disodium salt of ATP and 250 mg sodium hydrogen carbonate in 5.0 mL water.

NOTE: This solution is stable for 4 weeks at 4°C or 2 months at –20°C.

(i) *HK/G-6-P-DH mixture*, containing 2 mg hexokinase (HK) and 1 mg glucose-6-phosphate dehydrogenase (G-6-P-DH) per millilitre.

NOTE: This enzyme mixture is stable for 1 year at 4°C.

6 APPARATUS

In addition to usual laboratory apparatus, the following apparatus is required:

(a) Spectrophotometer capable of reading absorbance at 340 nm with 10 mm cells.

(b) Volumetric dispensers (for example, piston operated volumetric apparatus (POVAs)) capable of delivering the volumes required to an accuracy better than ±1.0 percent and repeatability better than ±0.5 percent.

7 SAMPLES AND SAMPLE PREPARATION

7.1 Sampling

Ensure samples taken for analysis are typical or representative of bulk lot.