# Australian Standard®

# Methods of chemical and physical testing for the dairying industry

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# 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) Sucrose + H<sub>2</sub>O 
$$\xrightarrow{\text{fructosidase}} \beta$$
 glucose + fructose.

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

(c) Glucose-6-phosphate + NADP<sup>+</sup>  $\longrightarrow$  6-phosphogluconate + NADPH + H<sup>+</sup>.

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.