

Australian Standard®

AS 2300.1.8—2008

Methods of chemical and physical testing for the dairying industry

Method 1.8: General methods and principles— Assessment of instrumental methods—Protein in milk and infrared spectrometric analysis of milk

PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Subcommittee FT-024-05, Dairy Products, to supersede AS 2300.1.8.1, *Methods of chemical and physical testing for the dairying industry—Method 1.8.1: General, methods and principles—Analytical quality assurance of instrumental methods—Protein in milk* and AS 2300.1.8.2, *Methods of chemical and physical testing for the dairying industry—Method 1.8.2: General methods and principles—Assessment of instrumental methods—Infrared spectrometric analysis of milk*.

After a periodic review, the Committee recommended to amalgamate two parts into one Standard to provide procedures to assess the capabilities of instrumental methods for the determination of protein in milk. This edition confirms the method without technical changes, but updates the referenced documents and reflects the current editorial style. It 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

The increasing use of instrumental methods for estimation of the composition of milk has made it necessary for a standard procedure to be devised to assess the capabilities of instrumental methods compared with accepted standard chemical methods.

The purpose of Section 2 of this Standard is to provide a means by which the protein content of liquid milk from an instrumental method can be related to the results obtained by a standard chemical method. When reporting results obtained by an instrumental test method, the analyst may quote that the instrument has been validated in accordance with this Standard.

Section 3 of this Standard deals with the assessment of the performance of mid-infrared instruments for analyzing milk.

This Standard makes no attempt to describe routine procedures for their operation; this is left to the manufacturer's manual of instructions and regulatory or local requirements for calibration, with the expectation that good laboratory practice will be used. Further information on routine procedures for operation of the instruments is also given in International Dairy Federation (IDF) Provisional Standard 141 (Ref. 1). The assumption is also made that the instruments are designed to operate on sound physical and chemical principles. While instrumental methods are less susceptible to operator bias, a rigid discipline of check procedures as described in the manufacturer's manual is essential to ensure that the instrument functions at all times within its design limits.

METHOD

SECTION 1 SCOPE AND GENERAL

1.1 SCOPE

This Standard describes a procedure to assess the capabilities of instrumental methods with accepted standard chemical methods.

1.2 REFERENCED DOCUMENTS

The following Standards are referred to in this Standard.

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| 2300 | Methods of chemical and physical testing for the dairying industry |
| 2300.1.2.1 | Method 1.2.1: General methods and principles—Determination of nitrogen—Reference Kjeldahl method |
| 2300.1.2.2 | Method 1.2.2: General methods and principles—Determination of nitrogen—Nitrogen fractions from milk |
| 2300.1.3 | Method 1.3: General methods and principles—Determination of fat—Gravimetric method |
| 2300.2.6 | Method 2.6: Liquid milks—Determination of lactose |

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.

1.3 ACCURACY UNCERTAINTY OF MEASUREMENT

Estimate the uncertainty of test measurements based on the performance characteristics of the test method (trueness and precision), verification data, quality control data, previous experience or other relevant information.

The appropriate degree of rigour applied to estimation of uncertainty depends on the purpose of the test and customer (end-user) requirements.

NOTES:

- 1 An estimate of uncertainty is required when it is relevant to the validity or application of the test result or when requested by a customer.
- 2 Unless otherwise required, the measurement value should be stated together with the expanded uncertainty, calculated using a coverage factor of 2, to give a level of confidence of approximately 95%.
- 3 The measurement value and its uncertainty should both be reported in the same units, the measurement value rounded to be consistent with its uncertainty.
- 4 The form of reporting the result must not give a wrong impression of the uncertainty.

SECTION 2 PROTEIN IN MILK

2.1 SCOPE

This Section describes a procedure for evaluating the suitability of an individual instrument for measuring protein in liquid milk.

NOTE: This is intended for instruments with multi-component capability such as those that can also determine fat and lactose. However, the Section does not validate the instrument's capabilities for measuring analytes other than protein.

2.2 APPLICATION

The procedure is applicable to all instruments capable of analysing for both crude and true protein (see Clause 2.3) in liquid milk. It is intended to verify that an instrument is capable of performing a single analysis on a sample of milk to produce a result of comparable precision and accuracy to that obtained when the protein content of the milk is estimated from the nitrogen content, determined by the reference Kjeldahl method set out in AS 2300.1.2.1.

2.3 DEFINITIONS

For the purpose of this Standard, the following definitions apply.

2.3.1 Crude protein

The total nitrogen content of the sample multiplied by the factor 6.38.

2.3.2 True protein

The total nitrogen content of the sample minus the non-protein nitrogen content and multiplied by the factor 6.38.

NOTE: The total nitrogen and non-protein nitrogen contents are determined by the methods set out in AS 2300.1.2.2.

2.4 PRINCIPLE

After the instrumental performance checks, adjustments and calibrations have been carried out according to the manufacturer's instructions, suitable samples of milk of the type to be analysed are measured for protein content by both the instrument and the reference Kjeldahl procedure. The results of both methods are compared by a defined statistical procedure. The conformance of the instrument is judged by defined criteria.

2.5 TEST SAMPLES

Samples used in the comparison should be of the type to be measured. They should be prepared as described by the manufacturer for the particular instrument, and as appropriate for Kjeldahl analysis.

2.6 COMPARISON OF INSTRUMENT WITH REFERENCE METHOD

2.6.1 General

It is essential that all steps in the procedure are carried out on the same day. It must be known that the instrument is performing correctly before its accuracy is assessed.

2.6.2 Instrument performance checks

Before calibrating the instrument, checks of zero settings, linearity, purging efficiency and repeatability are performed. Make any adjustments necessary for correct performance of the instrument according to the manufacturer's instructions.

NOTE: Appendix A gives recommended methods for checking repeatability and purging efficiency.

2.6.3 Instrument calibration

Calibrate the instrument according to the manufacturer's instructions using milk samples of the type to be measured.

2.6.4 Comparative analyses

The procedure shall be as follows:

- (a) Analyse in duplicate a set of at least eight prepared samples covering the range of protein content normally measured, using the reference Kjeldahl method set out in AS 2300.1.2.1. Use the same procedures as those used to obtain the reference values with which the instrument is calibrated.
- (b) Analyse the samples by the instrumental method, in ascending order of protein content. Repeat the instrumental analysis in descending order of protein content.

NOTES:

- 1 The first test (possibly more) made with the instrument after a rest period may give an incorrect result.
- 2 The first test (possibly more) made with the instrument after it has been used for a sample with a widely different protein content, may give a result that is biased towards that of the previous sample.
- 3 More accurate results can be obtained with the instrument by repeating the tests on a sample until there is no drift in the readings, and by then taking the average of a number of subsequent readings.

2.7 CALCULATIONS

2.7.1 Mean difference

Calculate the mean difference (*MD*) between reference and instrument results as follows:

$$MD = \frac{\sum D}{n} \quad \dots 2.7(1)$$

where

D = the difference between the average reference result and each single instrument result for each of the samples tested

n = the number of instrument tests

2.7.2 Standard deviation

Calculate the standard deviation of difference between reference and instrument results *S_D(diff)* from the following equation:

$$S_D(\text{diff}) = \sqrt{\frac{\sum D^2 - (\sum D \times MD)}{n - 1}} \quad \dots 2.7(2)$$

where *D*, *n* and *MD* are shown in Clause 2.7.1.

2.8 CRITERIA FOR ACCEPTABILITY OF AN INSTRUMENT

An instrument is considered to be acceptable, i.e. provides results which are comparable with the reference method AS 2300.1.2.1, if it meets the following criteria resulting from the tests carried out in accordance with this Standard:

- (a) Mean difference between instrument results and reference results is not more than 0.05 percent protein.
- (b) Standard deviation of difference between instrument results and reference results is not more than 0.06 percent protein.

2.9 REPORT

When reporting the use of this Standard for assessing the compliance of an instrument, include the following details:

- (a) The brand and model of instrument used.
- (b) The nature of the samples used in the tests.
- (c) Reference to this Australian Standard, i.e. AS 2300.1.8.
- (d) The reference method used to determine protein values, i.e. AS 2300.1.2.1.
- (e) The results determined as true or crude protein (see Clause 2.3).
- (f) Where applicable, a statement concerning the estimated uncertainty of measurement.
- (g) Date of the tests performed.
- (h) Any circumstance or unusual observations made during the course of the test which may have had an effect on the result.

2.10 BIBLIOGRAPHY

Sections 972.16A to 972.16G, *Official Methods of the Association of Official Analytical Chemists*, 18th ed., 2005, AOAC Inc. Arlington, Virginia, USA.