Australian Standard®

Methods of chemical and physical testing for the dairying industry

Method 1.2.2: General methods and principles-Determination of nitrogen—Nitrogen fractions from 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 2300.1.2.2—1988.

The Committee did not recommend the adoption of ISO 8968-4 and ISO 8968-5, because these two ISO Standards do not cover all four fractions of nitrogen of milk. This Standard covers all four fractions of milk, i.e: non-protein nitrogen, non-casein nitrogen, casein nitrogen and non-casein protein nitrogen, which are required for an Australian Standard.

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 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

STANDARDS Australia

METHOD

1 SCOPE

This Standard sets out procedures for obtaining various fractions from milk and determining the following:

(a) Non-protein nitrogen.

(b) Non-casein nitrogen.

(c) Casein nitrogen.

(d) Non-casein protein nitrogen.

2 APPLICATION

The methods are applicable to milk, skimmed milk and recombined or reconstituted milk.

3 REFERENCED DOCUMENT

The following documents are referred to in this Standard.

AS
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.

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.

4 PRINCIPLE

Milk protein fractions are precipitated from milk by various reagents and the supernatant liquids are filtered. The nitrogen content of the filtrate is determined and, in some cases, the nitrogen content of the precipitate is determined mathematically by difference.

5 REAGENTS

Use only reagents of recognized analytical reagent grade, and freshly distilled water or water of equivalent purity. The following reagents are required:

(a) Reagents listed in AS 2300.1.2.1.

(b) Trichloracetic acid solution, 15%: dissolve 15 g trichloracetic acid in water and make up to 100 mL.

(c) Acetic acid solution, 10%: add 10 g glacial acetic acid to 50 mL of water and make up to 100 mL

(d) Sodium acetate, 1 molar solution: dissolve 8.203 g anhydrous sodium acetate in water and make up to 100 mL.

6 APPARATUS

The following apparatus is required:

(a) Apparatus listed in AS 2300.1.2.1.

(b) 100 mL one-mark volumetric flasks.

(c) Whatman No. 40 filter papers of 11 cm diameter.

7 SAMPLES

The sample used for analysis of milk fractions shall be the same as that used for total nitrogen determination (see Clause 8.2).

8 PROCEDURES

8.1 General

The nitrogen contents of the original sample and of the filtrates (see below) shall be determined by the Kjeldahl method described in AS 2300.1.2.1.

8.2 Total nitrogen

Determine the total nitrogen (*TN*) content of the sample in percent *m/m.*

8.3 Non-protein nitrogen

The procedure shall be as follows:

(a) Weigh 20 mL of sample (see Clause 7) to the nearest 10 mg (mass m_1) into a 100 mL one-mark flask.

(b) Dilute with 15% trichloracetic acid solution and make up to the mark with this reagent.

(c) Stopper the flask and mix immediately.

(d) Allow the precipitate to settle.

(e) Filter the supernatant through a dry fluted 11 cm Whatman No. 40 filter paper into a dry flask.

(f) Determine the nitrogen content (N_1) of 50 mL (aliquot V_1) of the filtrate.

8.4 Non-casein nitrogen

The procedure shall be as follows:

(a) Weigh 40 mL of sample (see Clause 7) to the nearest 10 mg (mass m_2) into a 100 mL one-mark flask.

(b) Add 40 mL of water and warm the contents of the flask to 35° C.

(c) Add 4.0 mL of 10% acetic acid.

(d) After 10 min, add 4.0 mL of 1 molar sodium acetate solution.

(e) Allow to cool, make up to 100 mL with water and mix.

(f) Allow the contents to stand until a clear supernatant is formed.

(g) Filter the supernatant through a dry fluted 11 cm Whatman No. 40 filter paper.

(h) Determine the nitrogen content (N_2) of 20 mL (aliquot N_2) of the filtrate.

NOTE: Low values for non-casein nitrogen will be obtained if the milk has been severely heated e.g. reconstituted milk. This is due to the coprecipitation of some of the whey proteins as well as casein.