

Australian Standard®

AS 2300.1.3—2008

Methods of chemical and physical testing for the dairying industry

Method 1.3: General methods and principles— Determination of fat—Gravimetric method

PREFACE

This Standard was prepared by the Standard Australia Committee, FT-024, Food Products and Subcommittee FT-024-05, Dairy Products to supersede AS 2300.1.3—1988.

The Committee did not recommend the adoption of ISO Standards, which were outdated and product specific. The superseded ISO standards include a test for solubility of extracted material and, if insolubles are present, fat is calculated from the mass difference before and after the collection vessel is washed out. Current ISO standards rely totally on visual inspection of the extracted material. As such, the Committee did not recommend adopting the relevant ISO standards to replace AS 2300.1.3—1988, as the approach taken for final reporting differs between standards, and this approach is not suitable for a reference method.

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

SECTION 1 SCOPE AND GENERAL

1.1 SCOPE

This Section sets out a general gravimetric method for the determination of fat in dairy products.

NOTE: Methods of preparation of test samples of specific products for determination of fat content by this method are described in Section 2.

1.2 APPLICATION

A general method is given, and this is applicable to prepared samples for a range of dairy products.

1.3 REFERENCED DOCUMENTS

The following documents are referred to in this Standard.

AS

1152 Specification for test sieves

1166 Milk and milk products—Guidance on sampling

AS/NZS

2243 Safety in laboratories

2243.1 Part 1: Planning and operational aspects

2243.2 Part 2: Chemical aspects

ISO

383 Laboratory glassware—Interchangeable conical ground joints

3889 Milk and milk products—Specification of Mojonnier-type fat extraction flasks

1.4 DEFINITIONS

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

1.4.1 Fat content

The substances determined by the procedure described in this Standard.

1.4.2 Laboratory sample

A sample prepared for sending to the laboratory, and comprising a quantity of bulk product or a sufficient number of unopened retail containers to fulfil test requirements.

NOTE: It is assumed that the laboratory sample has been taken, transported to the laboratory, and stored in accordance with the requirements of AS 1166.

1.4.3 Test portion

The quantity of product actually drawn from the test sample (or, if both are the same, from the laboratory sample) and on which the test is actually carried out.

1.4.4 Test sample

A sample in the state of preparation in which it is subjected to the test.

SECTION 2 GENERAL METHOD

2.1 PRINCIPLE

The protein in the test portion is dissolved and the fat is freed for extraction by treatment with ammonia or acid solution. Ethanol is added to prevent emulsion formation and the fat is extracted with diethyl ether, after which light petroleum is added to eliminate the lactose and water from the ethereal layer. Solvents are removed by distillation or evaporation, and the mass of the extracted substances which are soluble in light petroleum is determined.

NOTE: Digestion with ammonia is in accordance with the Röse-Gottlieb principle. Digestion with acid is in accordance with the Schmid-Bondzynski-Ratzlaff principle.

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.

2.2 REAGENTS

2.2.1 General requirements

Use only reagents of recognized analytical reagent grade, and freshly distilled water or water of equivalent purity. Carry out the blank test described in Clause 2.4.1 on fresh batches of reagents. Do not use reagents which leave a residue of more than 1 mg when so tested.

WARNING: SOME OF THE REAGENTS ARE VOLATILE, FLAMMABLE SOLVENTS OF A TOXIC NATURE. AVOID CONTACT WITH THE SKIN AND INHALATION OF THE VAPOURS. OBSERVE THE NECESSARY PRECAUTIONS TO PREVENT FIRE, ESPECIALLY WHEN USING ELECTRICAL APPARATUS. SEE AS/NZS 2243 FOR MORE DETAILS REGARDING LABORATORY SAFETY.

2.2.2 Reagents

The following reagents are required:

- (a) Ethanol, 93% to 97% *V/V*

NOTE: Ethanol may be replaced by methylated spirits called Industrial Spirits complying with Part IV of the Commonwealth of Australia Spirits Regulations in force under the Spirits Act. Special methylated spirit (ethanol denatured with 2% methanol only) or Spirit SVR (95% to 96% ethanol) may be used.

- (b) Congo-red solution. Dissolve 1 g Congo-red in water and dilute to 100 mL.

NOTE: This solution may optionally be used in the Röse-Gottlieb method. It allows the interface between the solvent and aqueous layers to be seen more clearly.

- (c) Diethyl ether, peroxide free, of boiling range 34°C to 35°C.

NOTE: To test for peroxides, rinse a small glass-stoppered cylinder with water, add 10 mL of the ether and 1 mL of a freshly prepared potassium iodide solution (10 g/100 mL). Shake the mixture and allow to stand for 1 min. The development of a yellow colour in either layer indicates the presence of peroxides. Alternately, appropriate test strips may be used.

- (d) Light petroleum (petroleum ether), boiling within the range 30°C to 60°C. Alternatively, hexane may be used.

- (e) Mixed solvent, prepared shortly before use by mixing equal volumes of diethyl ether and light petroleum.

2.3 APPARATUS

The following apparatus is required:

- (a) Fat extraction tubes, Mojonnier type, as listed in Clause 3.3(a).
- (b) Centrifuge, in which the fat-extraction tubes can be spun at 500 r/min to 600 r/min to produce a gravitational field of 80 g to 90 g at the outer end of the tubes.
NOTE: The use of the centrifuge is optional, but recommended (see Clause 2.4.3(c)).
- (c) Fat-collecting vessels, e.g. boiling flasks (flat-bottomed) or conical beakers, capacity 250 mL. Metal dishes may be used for the Röse-Gottlieb method.
- (d) Distillation or evaporation apparatus, to enable the solvents and ethanol to be distilled from the flasks, or to be evaporated from the beakers or dishes, at a temperature not exceeding 100°C.
- (e) Drying oven, electrically-heated, with natural or mechanically assisted convection.

2.4 PROCEDURE

2.4.1 Blank test

At the same time as the determination of the fat content of the sample, perform a blank determination using the method described below, using the blank prepared in Section 3, Clause 3.4.2. If the blank exceeds 1 mg, the reagents should be checked and the impure reagent or reagents purified or replaced.

2.4.2 Test samples and digestion

Refer to Section 3 for details of preparation of test samples and their digestion.

2.4.3 Analytical method

The procedure shall be as follows:

- (a) To the digest in the Mojonnier tube prepared according to Section 3, add 10 mL of ethanol and mix gently but thoroughly by allowing the contents of the tube to flow backward and forward between the two bulbs. Avoid contact between the liquids and the neck of the tube.

NOTES:

- 1 If desired, when using the Röse-Gottlieb method, two drops of Congo-red solution may be added (see Note to Clause 2.2.2(b)).
 - 2 Complete extraction of the fat depends on satisfactory mixing at each stage.
 - 3 It is necessary to wet the stopper with water each time it is inserted into the Mojonnier tube and to wash it with solvent, as directed, during each removal. Also, before each removal, a slightly reduced pressure should be induced in the flask by cooling it, to avoid spurting of the solvent.
- (b) Cool the Mojonnier tube, add 25 mL of diethyl ether, stopper the tube and mix by swirling. Shake vigorously but not excessively for 1 min with the tube in a horizontal position and the small bulb extending upwards. The tube should be shaken so that the liquids are moved rapidly back and forth along its length, and periodically the liquids should be allowed to run from the larger bulb into the smaller one.
 - (c) Wash the stopper and neck of the Mojonnier tube with 25 mL of light petroleum so that the washings run back into the tube. Replace the stopper and shake as before for 30 s. Centrifuge the tube until the ethereal layer is clear, or alternatively allow to stand at least 30 min until the ethereal layer is clear.