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Milk — Determination of fat content

Lait — Détermination de la teneur en matière grasse

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 2446 | IDF 226 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This second edition of ISO 2446 | IDF 226 cancels and replaces the first edition (ISO 2446:1976), of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

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ISO 2446 | IDF 226 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the former Joint ISO/IDF/AOAC Group of Experts (E40-E301) which is now part of the Joint ISO-IDF Action Team on *Fat*, of the Standing Committee on *Main components in milk.*

Milk — Determination of fat content

1 Scope

This International Standard specifies the Gerber method for the determination of the fat content of milk and includes guidance on the determination of the appropriate capacity of the milk pipette and on the determination of the corrections to apply to the results if the milk is not of average fat content (see 6.1). The procedure for checking the capacity of the milk pipette is specified in Annex A.

The method is applicable to liquid milk, whole or partially skimmed, raw or pasteurized. With modifications, details of which are given, it is also applicable to:

a) milk containing certain preservatives (see Clause 11);

b) milk that has undergone the process of homogenization, in particular sterilized milk and ultra heat-treated (UHT) milk (see Clause 12);

c) skimmed milk (see Clause 13).

NOTE The result obtained by the procedure specified in Clause 12 (modified for milk that has undergone homogenization) may be slightly high.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 488 | IDF 105, *Milk — Determination of fat content — Gerber butyrometers*

ISO 1211 | IDF 1, *Milk — Determination of fat content — Gravimetric method (Reference method)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
Gerber method
empirical procedure which gives a value for fat content either as a mass fraction or as a mass concentration — depending on the capacity of the milk pipette used — that is the same as, or has a known relationship to, the value obtained by the reference method specified in ISO 1211 | IDF 1

NOTE The mass fraction is expressed in grams of fat per 100 g of milk and the mass concentration in grams of fat per 100 ml of milk.

4 Principle

The milk fat in a butyrometer is separated by centrifuging after dissolving the protein with sulfuric acid, the separation being aided by the addition of a small quantity of *iso*-amyl alcohol. The butyrometer is graduated to give a direct reading of fat content.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and only distilled or demineralized water or water of equivalent purity.

5.1 Sulfuric acid

5.1.1 Requirements

The sulfuric acid shall have a density at 20° C of (1,816 ± 0,004) g/ml, which corresponds to approximately (90,4 ± 0,8) % mass fraction H_2SO_4. The acid shall be colourless, or not darker in colour than pale amber, shall be free from suspended matter and shall be found suitable for use when tested as specified in 5.1.2.

5.1.2 Suitability test

5.1.2.1 Purpose of test

Sulfuric acid can satisfy the specific requirements of 5.1.1 for density and appearance and yet be unsuitable for the Gerber method. Therefore, check the suitability of the acid before use by means of the following comparative test with a standard sulfuric acid.

5.1.2.2 Standard sulfuric acid

Add sulfuric acid [e.g. $w(H_2SO_4) = 98$ % mass fraction; $\rho_{20} = 1{,}84$ g/ml] to water to obtain a solution with a density within the range specified in 5.1.1.

NOTE Approximately 1 l of standard sulfuric acid is obtained by adding 908 ml of 98 % mass fraction sulfuric acid to 160 ml of water, checking the density of the diluted acid with a suitable hydrometer and adjusting the density, if necessary, by adding a small volume of water or 98 % mass fraction acid.

5.1.2.3 Comparison procedure

Determine in duplicate the fat content of four samples of whole milk with average fat content by the Gerber method specified, using butyrometers whose scale errors are less than 0,01 % and standard *iso*-amyl alcohol (5.2.6.2). In one of each pair of duplicates use 10 ml of the sulfuric acid under test and in the other use 10 ml of the standard sulfuric acid (5.1.2.2). Keep the butyrometers in a random order from the shaking stage onwards. Take the readings to the nearest 0,01 % fat (read by at least two persons). The mean fat content of the four milk samples obtained with the sulfuric acid under test shall not differ by more than 0,015 % fat from the mean value obtained using the standard sulfuric acid.

5.2 *Iso*-amyl alcohol

5.2.1 Composition

A volume fraction of at least 98 % of the "*iso*-amyl" alcohol shall consist of the primary alcohols 3-methylbutan-1-ol and 2-methylbutan-1-ol, the only permissible major impurities being 2-methylpropan-1-ol and butan-1-ol. It shall be free from secondary pentanols, 2-methylbutan-2-ol, furan-2-al (furfural, furan-2-carboxaldehyde,
2-furaldehyde), gasoline (petrol) and derivatives of benzene. Not more than a trace of water shall be present.

5.2.2 Physical appearance

The *iso*-amyl alcohol shall be clear and colourless.

5.2.3 Density

The *iso*-amyl alcohol shall have a density at 20 °C of 0,808 g/ml to 0,818 g/ml.

5.2.4 Furan-2-al and other organic impurities

When 5 ml of the *iso*-amyl alcohol is added to 5 ml of the sulfuric acid (5.1), no more than a yellow or light-brown colour shall develop.

5.2.5 Distillation range

When the *iso*-amyl alcohol is distilled at a pressure of 101,3 kPa[1)], a volume fraction of not less than 98 % shall distil below 132 °C and a volume fraction of not more than 5 % below 128 °C. There shall be no solid residue after distillation.

If the atmospheric pressure during the distillation is lower or higher than 101,3 kPa, the specified temperatures should be decreased or increased, respectively, by 0,3 °C/kPa.

5.2.6 Suitability test

5.2.6.1 Purpose of test

An *iso*-amyl alcohol can satisfy the requirements of 5.2.1 to 5.2.5, yet be unsuitable for the Gerber method. Therefore, check the suitability of the *iso*-amyl alcohol before use by means of the following comparative test with a standard amyl alcohol.

5.2.6.2 Standard *iso*-amyl alcohol

Distil an *iso*-amyl alcohol satisfying the requirements of 5.2.1 to 5.2.5, using a suitable fractionation column, and collect a fraction within a boiling range of 2 °C between 128 °C and 131,5 °C (see 5.2.5, paragraph 2). Apply the following tests to the fraction:

a) when analysed by gas-liquid chromatography, a volume fraction of at least 99 % shall consist of 3-methylbutan-1-ol and 2-methylbutan-1-ol — only traces of impurities other than 2-methylpropan-1-ol and butan-1-ol shall be present;

1) 1 kPa = 10 mbar.

b) when fractionally distilled, the first 10 % and the last 10 % collected, when compared using the procedure specified in 5.2.6.3, shall give values for the fat content of milk that do not differ by more than 0,015 % fat.

If the fraction satisfies both these tests, it can be regarded as standard *iso*-amyl alcohol. The standard *iso*-amyl alcohol can be used for several years, provided that it is kept in the dark in a cool place.

5.2.6.3 Comparison procedure

Determine in duplicate the fat content of four samples of whole milk with average fat content by the Gerber method specified, using butyrometers whose scale errors are less than 0,01 % and standard sulfuric acid (5.1.2.2). In one of each pair of duplicates, use 1 ml of the *iso*-amyl alcohol under test, and in the other use 1 ml of the standard *iso*-amyl alcohol (5.2.6.2).

Keep the butyrometers in a random order from the shaking stage onwards. Take the readings to the nearest 0,01 % fat (read by at least two persons).

The mean fat content of the four milk samples obtained with the *iso*-amyl alcohol under test shall not differ by more than 0,015 % fat from the mean value obtained using the standard *iso*-amyl alcohol.

Instead of the *iso*-amyl alcohol specified, an artificial *iso*-amyl alcohol or an *iso*-amyl alcohol substitute, coloured if desired, may be used, provided that its use has been demonstrated by experiment not to lead to any significant differences in the results of the determination.

6 Apparatus

6.1 Milk pipette.

6.1.1 The milk pipette shall be of the single graduation line, bulb type, and its capacity shall be defined as the volume, in millilitres, of water at 20 °C (27 °C in tropical countries) delivered by the pipette when emptied as specified in Annex A.

The capacity of the pipette, determined by the method specified in Annex A, shall not differ from the nominal capacity, established according to 6.1.3, by more than 0,03 ml.

6.1.2 The capacity of the milk pipette shall be such that, when the pipette is used as specified in 9.2 (i.e. using the top of the milk meniscus when adjusting the milk to the graduation line) and whichever method of expressing the result is adopted (see Clause 3), the value for fat content obtained agrees with the value obtained by the reference method using whole milk having a fat content equivalent to the accepted average of the national milk supply.

Some milk pipettes are available which allow the bottom of the milk meniscus to be observed during pipetting. If such pipettes are used, their capacity should be such that when they are used with milk of average fat content the requirement of the preceding paragraph is satisfied.

6.1.3 In each country, the appropriate capacity (see 6.1.1 and 6.1.2) of the milk pipette shall be established by carrying out comparative determinations using the Gerber method specified and the reference method specified in ISO 1211 | IDF 1 on a large number of whole milks covering a wide range of fat content. Statistical analysis of the results of these determinations, taken in conjunction with a knowledge of the national average fat content of milk shall be used to establish the appropriate capacity of the milk pipette. These comparative determinations on whole milk, together with similar determinations on partially skimmed milk and skimmed milk, will also provide the corrections to be applied, if desired or if necessary, to Gerber values when milk is not of average fat content. For these comparative determinations, butyrometers whose scale errors are less than 0,01 % shall be used, and the butyrometers read to the nearest 0,01 % fat.

6.1.4 If the value for fat is to be expressed in grams of fat per 100 ml of milk, the basis of comparison with the reference method shall be stated.

6.2 Butyrometer and **stopper**, as specified in ISO 488 | IDF 105.

Use a butyrometer whose scale range is appropriate for the expected fat content of the sample. In the case of skimmed milk, use a 0 % to 0,5 % butyrometer.

With corrugated-neck butyrometers, either lock stoppers or solid single-ended or double-ended rubber stoppers may be used.

With plain-neck butyrometers, lock stoppers should preferably be used.

6.3 Automatic measure, or **safety pipette**, capable of delivering (10,0 ± 0,2) ml, and in the case of skimmed milk, (20,0 ± 0,2) ml, of sulfuric acid (5.1).

6.4 Automatic measure, or **safety pipette**, capable of delivering (1,0 ± 0,05) ml, and in the case of skimmed milk, (2,0 ± 0,2) ml, of *iso*-amyl alcohol (5.2).

6.5 Protected stand, for shaking the butyrometers (6.2).

6.6 Centrifuge, in which the butyrometers can be spun, provided with a rotational frequency indicator, graduated in revolutions per minute, with a maximum tolerance of ± 50 r/min, and preferably of the vertical-loading type rather than the horizontal-loading type.

The design of the centrifuge shall be such that the temperature of the butyrometer contents after the centrifuging (see 9.6) is between 30 °C and 50 °C.

When fully loaded, the centrifuge shall be capable of producing, within 2 min, a relative centrifugal acceleration of (350 ± 50)g at the outer end of the butyrometer stopper. This acceleration is produced by centrifuges with the effective radius (horizontal distance between the centre of the centrifuge spindle and the outer end of the butyrometer stopper) operated at the rotational frequency indicated in Table 1.

Table 1 — Centrifuge effective radius and rotational frequency to produce centrifugal acceleration of (350 ± 50)g

Effective radius mm	Revolutions per minute ± 70 r/min
240	1 140
245	1 130
250	1 120
255	1 110
260	1 100
265	1 090
270	1 080
275	1 070
300	1 020
325	980

NOTE The relative centrifugal acceleration produced in a centrifuge is given by Formula (1):

$$1{,}12rn^2 \times 10^{-6} \quad (1)$$

where

r is the effective horizontal radius, in millimetres;

n is the rotational frequency, in revolutions per minute.