INTERNATIONAL STANDARD

ISO 11870

First edition
2000-03-01

Milk and milk products — Determination of fat content — General guidance on the use of butyrometric methods

Lait et produits laitiers — Détermination de la teneur en matière grasse — Directives générales pour l'utilisation des méthodes butyrométriques

Reference number
ISO 11870:2000(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 734 10 79
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 11870 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*.

International Standard ISO 11870 has been prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations.

Annex A of this international Standard is normative, providing additional information concerning the limitation of the butyrometric methods specified.

Introduction

The reference methods described to determine the fat content of milk and milk products are time-consuming to apply and require some experience if reliable results are to be obtained.

Butyrometric techniques, which are simpler to apply, make it possible to obtain fat contents for various milk products quickly. This is why they are used in a great number of industrial laboratories as a fast method for routine checks.

Two acid-butyrometric methods used in many countries to determine the fat content of milk (Gerber method) and of cheese (Van Gulik method) are the subject of International Standards. The apparatus has also been standardized (ISO and IDF).

In addition, there are other butyrometric methods and butyrometers which have been described or applied in various countries for other types of products (cream, milk powder, etc.).

Whilst only one procedure exists as a reference method for a particular product type, this is not the case for butyrometric methods. Depending upon the country, different butyrometric methods may exist for one single type of product, presenting many problems for the harmonization of such procedures.

The second problem relates to the applicability of such methods. Indeed, with evolving manufacturing technologies, the variety of milk products is such that it is not possible to determine a method which can be applied to all varieties of a single type of product (milk, cheese, cream, etc.). Tests have confirmed this and have shown that the butyrometric methods already standardized have been attributed fields of application which are far too wide ranging.

Thus this general guide has been prepared while maintaining the existing standards.

Milk and milk products — Determination of fat content — General guidance on the use of butyrometric methods

1 Scope

This International Standard gives guidance on the following subjects:

— existing standardized methods (both reference and butyrometric) for the determination of the fat content of the various milk products;

— the principles underlying any acid-butyrometric analysis and the main operating requirements;

— a validation procedure for a butyrometric method in relation to the relevant reference method.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 2446:1976, *Milk — Determination of fat content (Routine method).*

ISO 3433, *Cheese — Determination of fat content — Van Gulik method.*

3 Principle

The principles of any butyrometric method remain constant independent of the product to be analysed. Protein is digested with sulfuric acid. The fat in the product is separated by centrifuging it in the butyrometer. The separation is enhanced by the addition of a small quantity of amyl alcohol. Direct reading of the butyrometer scale with or without correction.

4 Methods for the determination of fat content

Methods for the determination of fat content are based upon acid-butyrometric and reference gravimetric methods.

The Gerber method is specified in ISO 2446 and the Van Gulik method in ISO 3433. Existing butyrometric and reference methods for most dairy products are listed in Table A.1.

5 Reagents

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

5.1 Sulfuric acid, pure, colourless or pale amber and containing no impurities.

5.2 Amyl alcohol (1-pentanol), free of any secondary pentanol, 2-methylbutan-2-ol, 2-furfuraldehyde, gasoline (petrol) and derivatives of benzene.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Butyrometer and **stopper**, suitable for the method used.

6.2 Dispensers, for acid and alcohol, to deliver the requisite volumes precisely and with sufficient repeatability.

6.3 Centrifuge, capable of spinning a butyrometer, provided with a speed indicator which indicates the rotational frequency with a maximum tolerance of ±70 r/min, preferably of the vertical-loading type rather than the horizontal-loading type.

The centrifuge should be capable of maintaining the temperature of the butyrometer contents at between 30 °C and 50 °C after centrifuging.

NOTE The use of a heated centrifuge is permitted provided that the results obtained agree with the reference method.

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

Table 1 — Centrifuge accelerations

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

The relative centrifugal acceleration produced in a centrifuge, A_c, is given by the following formula:

$$A_c = 1{,}12 \times 10^{-6} \times RN^2$$

where

R is the effective horizontal radius, in millimetres;

N is the rotational frequency, in number of revolutions per minute.

6.4 Pipette or analytical balance, precise enough to ensure accurate distribution when preparing the test sample.

6.5 Water bath, thermostatically controlled, capable of maintaining the whole apparatus at the desired uniform temperature, and offering sufficient depth for the butyrometers to be supported in a vertical position with their scale graduations completely immersed.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

It is important the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

For each product to be analysed, refer to the relevant reference method.

9 Procedure

Accurately and quickly take a test portion from a homogeneous sample. Prepare the test portion by dissolving the protein by shaking, noting the type of shaking (vertical or horizontal, frequency and amplitude, etc.).

Centrifuge for a specified time with a specified centrifugal force. Take readings rapidly, immediately on removal from the water bath. If the fat cools, its volume decreases and the results obtained will be wrong.

If readings are being taken by hand, hold the butyrometer vertically with the point of reading at eye level. During this process, hold the stopper absolutely still.

If the fat is turbid or dark in colour, or if there is white or black material at the bottom of the fat column, the value for fat content will not be reliable.

If phase separation is not clear-cut, centrifuging twice would produce too high a result. In such cases, repeat the analysis.

10 Care of butyrometers

After the reading has been taken, the butyrometers should be inverted, stoppers upwards, on a rack. In approximately 30 min, the fat from the bulb and the graduation tube will rise upward under the stopper. As the butyrometers are still hot, remove the stoppers carefully, holding the open end close to the bottom of a sink.

The emptied butyrometers, still being hot, should be washed without use of a bottle brush, by shaking them vigorously with an appropriate detergent. The butyrometers should be plunged into water containing a detergent and filled and emptied several times and shaken vigorously, special attention being paid to the small bulb.

Then the butyrometers should be rinsed three times with hot water (i.e. three separate amounts, vigorously shaking and emptying each time).

Finally, the butyrometers should be shaken out very vigorously and allowed to drain with their open end downwards. They may be used again immediately, whilst still damp. However, it is important to shake them out again, immediately prior to use, in order to remove to the maximum extent any water droplets still inside.

11 Validation principle for a butyrometric method by comparison with the corresponding reference method

Whatever the butyrometric method used and whatever the product analysed, the method is only an empirical one. The result obtained must be comparable with the result obtained by the reference method. All laboratories, therefore, should validate their butyrometric methods by comparison with the corresponding reference method.

Adjusting the results from the acid-butyrometric method to those of the reference method is accomplished by varying a number of parameters and especially the following:

— concentration of the acid;

— temperature of the water bath;

— the physical properties of the butyrometer, such as the volume of the large bulb, the length and/or width of the graduated tube, the form of the graduated tube and the graduation scale.

The criterion for optimal adjustment is the absolute difference between the result obtained with the (modified) routine method and the result from the reference method, which should be minimized.

Once a set of conditions for the routine method has been found that gives equivalent results, the equivalence should be confirmed by comparing duplicate determinations with the two methods on several samples. The results from each set of samples can be compared using the classical Student's *t*-test.

CAUTION — This test presupposes that the variances of the two methods are equal. This should be checked in case of doubt.

As the optimal conditions found may be only valid for a limited concentration range of the analyte, the whole range for which the routine method will be used should be tested. Analysing in duplicate can do this by both methods (routine and reference) with samples spanning the whole range of fat contents. The equivalence of both methods should then be established by comparing the results for each sample using the *t*-test. If necessary, the results from testing the whole range can be used to establish a correction table (see also ISO 8196).

It should be stressed that, in laboratories which always verify the same type of product produced by the same process, the adjustment of the results obtained by the butyrometric method to the results obtained by the reference method shall be as perfect as possible. The absolute difference shall approximate to zero.

If a laboratory has to determine the fat content of products of the same type but of different origin, it will have problems in adjusting its butyrometric method so as to obtain a value identical in every case to that of a reference method.

Even for standardized butyrometric methods, regular checks are recommended because such methods, in addition to all the causes for variations already listed, have their own limitations (see annex A).

Annex A
(normative)

Limitations of butyrometric methods

A.1 Gerber method (see ISO 2446)

This method is applicable to raw or pasteurized, whole or partially skimmed, liquid milk and comprises modifications applicable to:

— milk containing preservatives;

— homogenized milk;

— skimmed milk.

However, it should be noted that:

— the volume of test portion to be used has never been internationally agreed, therefore it is not harmonized (whatever the volume of sample used, the result of the analysis must agree with the reference method; see also ISO 2446:1976, 6.1.2);

— the modified procedures for homogenized and skimmed milk have not proved satisfactory, therefore, several countries have developed their own procedures;

— the presence of added substances, such as sugar, flavourings, chocolate, etc., interferes with the results.

A.2 Van Gulik method (see ISO 3433)

This method is applicable to all types of cheese. However, it has been shown that the method is not wholly satisfactory for the following cheeses.

— Blue-veined cheeses: interference is produced by the presence of smaller or larger deposits at the base of the fat column.

— Long-matured cheeses: lipolysis alters the composition of the triglycerides, which distorts the fat content result obtained.

— Cheeses made from homogenized milks: results obtained are always too low.

— Low- or high-fat content cheeses: the results are not always in conformity with those obtained by the SBR reference method.

— Cheeses containing added substances: these may interfere and may agglutinate at the bottom of the column, producing too high results or too low results in comparison with the Weibull-Berntrop reference method.

— Cheeses made from milk other than cow's milk: the milkfat composition is different and, therefore, any butyrometric determination produces results which, to varying degrees, are wrong.

