INTERNATIONAL STANDARD

ISO 9622

IDF 141

Second edition
2013-09-15

Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry

Lait et produits laitiers liquides — Lignes directrices pour l'application de la spectrométrie dans le moyen infrarouge

Reference numbers
ISO 9622:2013(E)
IDF 141:2013(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received. www.iso.org/patents

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

The committee responsible for this document is 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 joint ISO 9622|IDF 141 cancels and replaces the first edition (ISO 9622:1999), which has been technically revised.

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 on 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.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

ISO 9622|IDF 141 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 an ISO-IDF Project Group on Guidance on the application of mid-infrared spectrometry, of the Standing Committee on *Statistics and Automation (SCSA),* under the aegis of its project leaders, Mr. P. Sauvé (CA) and Mr. H. van den Bijgaart (NL).

This second edition of joint ISO 9622|IDF 141 cancels and replaces IDF 141C:2000, which has been technically revised.

Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry

1 Scope

This International Standard gives guidelines for the quantitative compositional analysis of milk and liquid milk products, such as raw milk, processed milk, cream and whey, by measurement of the absorption of mid-infrared radiation.

Additional built-in instrument features, such as a conductivity sensor, can improve the performance in the determination of compositional parameters and allow for the estimation of other parameters.

The guidelines specified are applicable to the analysis of cow's milk. The guidelines are also applicable to the analysis of milk of other species (goat, ewe, buffalo, etc.) and derived liquid milk products, provided adequate calibrations are generated for each application and adequate control procedures are in place.

The application is limited to lower viscosity products that can be pumped through the flow system of the analyser and to analytes that do not result in optical saturation at the specific wavelengths being utilized.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable to its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8196|IDF 128 (all parts), *Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis*

ISO 8968-1|IDF 20-1, *Milk — Determination of nitrogen content — Part 1: Kjeldahl method*

ISO 8968-2|IDF 20-2, *Milk — Determination of nitrogen content — Part 2: Block-digestion method (Macro method)*

ISO 8968-5|IDF 20-5, *Milk — Determination of nitrogen content — Part 5: Determination of protein-nitrogen content*

NOTE Other normative documents can apply depending on the specific application or calibration of the automated analyser.

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8196|IDF 128 (all parts), and the following apply.

3.1
spectral calibration
spectrum calibration model
calibration based on combination of absorbance signals at several (>2) wavelengths in the mid-infrared region or signals from other sensors, mathematically optimized to arrive at the best estimate for the parameter of interest

3.2
slope and intercept calibration
simple linear regression coefficients as established from a least-squares regression of optimized instrument readings against results as obtained with physico-chemical reference methods

4 Principle

After pretreatment and homogenization, where required, the sample is measured with an infrared spectrometer that records the quantity of radiation absorbed in transmittance at specific wavelengths in the mid-infrared region. The spectral data are transformed into estimates of constituent concentrations or other physico-chemical parameters through calibration models developed on representative samples from the population to be tested. For some parameters, i.e. freezing point equivalents, signals from additional installed sensors may be fed to the calibration model.

5 Principal characteristics of infrared instruments

The signals at the relevant wavelengths may be produced using either a Fourier-transformed interferogram or by using optical filters. Instruments and applied calibration models may differ with respect to the number of specific wavelengths used in estimating the parameters of interest.

An infrared instrument is a proprietary apparatus which, when used under the conditions defined in this International Standard, provides estimates of compositional and other parameters in milk and liquid milk products.

6 Factors affecting the measurements

6.1 Instrument factors

6.1.1 Repeatability

To check instrument repeatability, analyse a uniform representative sample a minimum of 12 times in succession. The first two replicate results are discarded to minimize carry-over effects. The calculated repeatability should meet with the repeatability limits for the concerned parameter and sample matrix.

6.1.2 Zero stability

To monitor zero stability, a blank sample (water or zero solution) is analysed periodically during routine use of the instrument. Drift should be relatively small and random with respect to direction (±), such that cumulative drift is minimal. A plot of the zero drift vs time is an effective way to track instrument stability.

NOTE Certain instruments are factory set to auto-correct the zero at regular intervals. It is intended that operators review these automatic corrections to ensure that cumulative drift is not excessive.

6.1.3 Homogenization

To check the efficiency of the homogenizer, make two consecutive analyses, firstly with an unhomogenized whole milk sample, and secondly with the same whole milk sample after it has been homogenized through the instrument's homogenizer. When the average of five replicate fat readings is found, the difference among these five replicate fat readings shall not exceed 0,04 % for a milk sample containing a mass fraction of 4,0 % of milk fat. To calculate the appropriate pass/fail criteria for milk fat concentrations other than 4,0 %, multiply the actual fat content by 0,01 to obtain the new criteria.

NOTE 1 This procedure is only applicable to instruments in which the homogenized discharge can be isolated and collected.

NOTE 2 For applications involving sample matrices with higher levels of fat (i.e. raw cream), it is advisable to check homogenization efficiency with a representative high fat sample. Specific parameters for homogenizer performance depend upon the matrix.

NOTE 3 Instrument readings for every milk fat component (e.g. individual fatty acids or groups of fatty acids) is dependent on the effectiveness of homogenization. Different wavelengths used in calibration models result in unequal sensitivity to homogenizer efficiency and possibly larger relative effects than for fat. When measuring such milk fat components, it is intended that the homogenizer efficiency test be performed for these components, and the difference is not intended to exceed the limit of repeatability for the component.

CAUTION — The results of this test can be misleading, as an instrument in which the homogenizer does not work at all gives very little difference between the first and the second run.

An alternative procedure is to obtain an unhomogenized as well as a homogenized portion of the same milk, either by collecting raw and processed milk from the same tank at a dairy plant or by producing smaller volumes by means of a bench-top or pilot-plant homogenizer. Then measure both the unhomogenized and the same homogenized milk and compare the difference in results to the above-mentioned pass/fail criterion.

The assumption is that the homogenization efficiency of the external homogenizer is good. That can be verified by particle size analysis of the homogenized milk. A reasonable fat globule size distribution is characterized by a *d* (0,9) of 1,4 µm to 1,5 µm [*d* (0,9) means that 90 % of the milk fat globules has a diameter of less than *d*].[17]

Some instruments allow the user to monitor a homogenization index value to track the performance of the homogenizer. The manufacturer's guidelines should be followed.

Monitoring of instrument repeatability can also provide valuable information with respect to the state of the homogenizer. If repeatability on homogenized milk is satisfactory, whereas the repeatability on raw milk is poor (more than twice the variation), the homogenizer is likely not performing at an acceptable level.

6.1.4 Linearity

NOTE 1 The linearity check described in this subclause applies only to the measurement of major components in milk. Linearity checks for other applications, particularly for higher fat products or for parameters other than the major constituents, will differ. It is intended that the manufacturer's guidelines be followed in these cases.

NOTE 2 Linearity can be assessed on either a mass/mass basis or a mass/volume basis. Since the instrument cuvette holds a specific volume of sample, it is most ideal to assess linearity on a mass/volume basis. In either case, linearity solutions are prepared by accurately weighing fractions. To assess linearity on a volume basis, it is intended that accurate density measurements be conducted and appropriate conversions be calculated.

NOTE 3 It is critical, prior to assessing linearity, to confirm that the instrument homogenizer is functioning appropriately (see 6.1.3).

To check the linearity for each of the major components, make up at least 10 solutions of known concentration, which cover the typical range for the specific component. The following solutions are recommended.

a) Homogenized cream with a mass fraction of fat of 8 %, diluted with skimmed milk or zero solution to check the linearity for the determination of the fat content. If homogenized cream at this fat level is unavailable, unhomogenized cream may also be used providing the instrument homogenizer is functioning at an acceptable level (see 6.1.3).

b) UF skimmed milk retentate diluted with ultrafiltrate to check the linearity for the determination of the protein content. Alternatively, whey protein concentrate, sodium caseinate, calcium propionate, skim milk powder or evaporated skim milk diluted with distilled water may also be used. The stock solution should contain a mass fraction of approximately 5,5 % of protein.

c) A solution of 60 g/l of lactose monohydrate, diluted with water or a milk mineral solution[14] to check the linearity for the determination of the lactose content.

Using a stock solution, which has a concentration at the upper end of the typical range, serial dilutions can be made as follows in Table 1:

Table 1 — Serial dilutions

Part of stock solution	Part of diluent	Relative concentration
100	0	1,0
90	10	0,9
80	20	0,8
70	30	0,7
60	40	0,6
50	50	0,5
40	60	0,4
30	70	0,3
20	80	0,2
10	90	0,1
0	100	0,0

The concentrations of the solutions should be in regular increments from zero to the desired upper limits of instrument readings.

Analyse each sample in triplicate, average the results and calculate the linear regression equation $y = bx + a$. Apply linear regression with the expected values per sample on the x-axis and the measured values per sample on the y-axis. Calculate the residuals $e_i = y_i - (bx_i + a)$ from the regression. Plot the residuals e_i (y-axis) versus the expected values (x-axis) in a graph. A visual inspection of the data points usually yields sufficient information about the linearity of the signal. Any outlying residual should be deleted and the calculation process be repeated with the remaining data before applying the further test.

When observed, the curving can be expressed by the ratio, r, by using Formula (1):

$$r = \frac{(e_{max} - e_{min})}{(M_{max} - M_{min})} \times 100 \quad (1)$$

where

e_{max} is the numerical value of the maximum residual from the regression;

e_{min} is the numerical value of the minimum residual from the regression;

M_{max} is the numerical value of the upper measured value for the set of samples concerned;

M_{min} is the numerical value of the lower measured value for the set of samples concerned.

The ratio, r, should be less than 2 %. In case this value is superseded, better performance may be obtained by making separate calibrations for distinct ranges.

Eventually, adjust the linearity of the instrument response for the component in accordance with the manufacturer's instructions. See also Reference [18].

NOTE Alternatively, it is possible to combine a linearity check with the slope and intercept calibration.

6.1.5 Carry-over

Carry-over is defined as the residual volume of the previous sample as a percentage of the total volume of the instrument cell after a single pumping sequence of a sample through the instrument cell.

Internal factors/issues affecting carry-over include pump settings, flow system deficiencies and compensation factors. External factors affecting carry-over include transfer from the stirrer and pipette.

To assess carry-over for the complete system, including carry-over from the eventually applied automatic sampling system, run the samples from 20 separate vials using the complete system.

To assess carry-over for the flow system alone, run the samples manually, thereby wiping the pipette clean between cycles.

To check the carry-over, analyse 20 consecutive samples of water and whole (Be cautious with raw milk; it has to be homogenous.) milk, using the sequence: water, water, milk, milk, water, water, etc., and record for each sample of water and milk, the readings for each of the major compositional parameters.

Calculate for each parameter, the water-to-milk, E_W, and the milk-to-water carry-over, E_M, by using Formula (2) and (3):

$$E_w = \frac{(m_2 - m_1)}{(m_2 - w_2)} \times 100 \quad (2)$$

$$E_M = \frac{(w_1 - w_2)}{(m_2 - w_2)} \times 100 \quad (3)$$

where

w_1 is the sum of the first water readings (Nos. 1 + 5 + 9 + 13 + 17);

w_2 is the sum of the second water readings (Nos. 2 + 6 + 10 + 14 + 18);

m_1 is the sum of the first milk readings (Nos. 3 + 7 + 11 + 15 + 19);

m_2 is the sum of the second milk readings (Nos. 4 + 8 + 12 + 16 + 20).

The calculated carry-over values, E_W and E_M, shall be less than ±1 %.

NOTE It is intended that carry-over be assessed using this technique on the major milk components only.

6.1.6 Water vapour within the instrument

Variations in humidity of the air within the optical unit of the instrument result in variations in the optical zero and calibration. Replace the absorbent (silica gel) before it starts to change colour at the minimum interval specified by the manufacturer. The ambient conditions within certain laboratories might require changes that are more frequent.

6.2 Physico-chemical and biological factors

6.2.1 Milk composition

The signal obtained at each wavelength is the result of absorption by all components, including water.

When applying a spectrum calibration model for a specific component, the consequences of variations in other components may generally be accommodated for in the calibration model. Residual interaction detected can stem from insufficient variation of component concentrations in the spectral calibration sample set.

With traditional calibrations based on absorbance signals at preset wavelengths (MLR calibrations), it is necessary to apply intercorrection in order to accommodate for variations in concentration of the other components. The so-called intercorrection coefficients are specific to each wavelength and each type of instrument.