INTERNATIONAL STANDARD

ISO 9622

First edition
1999-11-15

Whole milk — Determination of milkfat, protein and lactose content — Guidance on the operation of mid-infrared instruments

Lait entier — Détermination des teneurs en matière grasse laitière, en protéines et en lactose — Lignes directrices pour l'utilisation des appareils de dosage par absorption dans le moyen infrarouge

Reference number
ISO 9622:1999(E)

Contents

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@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.

International Standard ISO 9622 was 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.

Annexes A, B and C of this International Standard are for information only.

Whole milk — Determination of milkfat, protein and lactose content — Guidance on the operation of mid-infrared instruments

1 Scope

This International Standard describes the operating conditions for instruments used for the determination of fat, protein and lactose content of ex-farm milk, based upon the measurement of the absorption of mid-infrared radiation at wavelengths which are representative of each component analysed.

NOTE 1 In practice, these measurements are made using commercial automatic or semi-automatic instruments defined in clause 5 and called "infrared instruments" in this International Standard.

Any model of instrument which does not comply with the principle of analysis given in this International Standard or which incorporates modifications that may alter the principal characteristics of the apparatus (repeatability, accuracy, conditions of use), as well as the means of adjusting the calibration, will require a separate specific standard.

NOTE 2 Not all instruments allow determination of the lactose content. Moreover, some instruments allow direct measurement of the water content. The total solids content can be estimated by adding together the fat, protein and lactose contents, a constant being used to correct for the salt content.

The method described is applicable to the determination of the fat, protein and, as appropriate, lactose content of ex-farm milk. The method is also applicable to the analysis of milk of other species (goat, ewe, buffalo, etc.) and processed milk provided a specific calibration of the instrument (see clause 7) is made.

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 agreement 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 1211, *Milk — Determination of fat content — Gravimetric method (Reference method).*

ISO 5765-1, *Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 1: Enzymatic method utilizing the glucose moiety of the lactose*.

ISO 5765-2, *Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 2: Enzymatic method utilizing the galactose moiety of the lactose*.

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

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

ISO 8968-4, *Milk — Determination of nitrogen content — Part 4: Determination of non-protein nitrogen content.*

ISO 8968-5, *Milk — Determination of nitrogen content — Part 5: Determination of protein-nitrogen content.*

3 Terms and definitions

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

3.1
infrared instrument
proprietary apparatus which, when used under the conditions defined in this International Standard, estimates the mass fraction of fat, protein and lactose in whole milk

3.2
fat, protein and lactose content
mass fraction of substances determined using the method specified in this International Standard

NOTE The fat, protein and lactose contents are expressed as mass fractions, in percent [formerly given as % (m/m)].

4 Principle

After homogenization of the milk sample, measurement with an infrared spectrometer of the quantity of radiation absorbed by:

— the carbonyl groups of the ester bonds of the glyceride at approximately 5,7 µm (traditionally referred to as the A Filter), and/or by the CH groups at approximately 3,5 µm (traditionally referred to as the B Filter), for determination of the fat content;

— the secondary amide groups of the peptide bonds at approximately 6,5 µm, for determination of the protein content;

— the hydroxyl groups of lactose at approximately 9,6 µm, for determination of the lactose content.

An estimate of the content of each component is made by reference to the amount of infrared light absorbed, either by water at the same wavelength or by the milk at a different wavelength at which there is only a slight absorption by the compound being measured.

NOTE For practical reasons, samples may be preserved with, for instance, a solution containing a mass fraction of 0,1 % potassium dichromate, 0,03 % sodium azide or 0,02 % to 0,06 % bronopol. It is necessary to check individual instrument response for all channels.

5 Principal characteristics of infrared instruments

The commercial instruments available may have one or two cells, with two wavebands or a single waveband per channel (component), and may use either a single- or a double-beam optical system, with either electronic ratioing or a servo system to estimate the transmitted radiation, and may produce the relevant wavelengths by diffraction grating, by interference optical filters, or by a Fourier-transformed interferogram. Instruments may differ also with respect to the number of specific wavelength ranges operating to predict the concentration of a given component.

NOTE In the case of interferometry, this International Standard is only applicable to the wavelength ranges mentioned in clause 4.

6 Factors affecting the accuracy of measurement

6.1 Instrument factors

6.1.1 Linearity

If an instrument is calibrated to express the results in mass/mass values, the solutions to adjust and evaluate linearity should be prepared on a mass/mass basis. If, on the other hand, the instrument is calibrated against volumetric reference methods or is calibrated to express results in mass/volume values, linearity should be set and evaluated on a mass/volume basis in order to give the optimal correlation to the reference method. Examples of how mass/volume evaluations can be carried out are given in annex A. This clause outlines only mass/mass based evaluations.

To check the linearity for each component, make up six solutions of known concentration as described in Table 1, using the following.

a) Unhomogenized cream is recommended with a mass fraction of fat of 8 %, diluted with skimmed milk, to check the linearity at wavelengths of 5,7 μm (A Filter) and 3,5 μm (B Filter) for the determination of the fat content.

b) UF skimmed milk retentate, diluted with ultrafiltrate is recommended to check the linearity at a wavelength of 6,5 μm for the determination of the protein content. Alternatively, whey protein concentrate, skim milk powder or evaporated skim milk diluted with distilled water may be used. The stock solution should contain a mass fraction of approx. 5,5 % protein.

c) A solution of 60 g/l of lactose monohydrate, diluted with water, is recommended to check the linearity at a wavelength of 9,6 μm for the determination of the lactose content.

Table 1

Parts of stock solution (i.e. cream, UF retentate or 6 % lactose solution)	**Parts of diluent** (i.e. skim milk or water)	**Relative concentration**
100	0	1,0
80	20	0,8
60	40	0,6
40	60	0,4
20	80	0,2
0	100	0

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

Whenever possible, use the primary signal to check the linearity. Analyse each sample in triplicate and calculate the linear regression equation, as follows:

$$y = bx + a$$

and residuals ε_i

$$\varepsilon_i = y_i - (ax_i + b)$$

Plot residuals ε_i (y-axis) versus the concentration of the component in solution (x-axis) on a graph for each of the components. A visual inspection of the data points will usually yield sufficient information about the linearity of the signal.

If a more objective criterion for linearity is required, calculate the ratio of the residual range to the range of signal values:

$$\Delta\varepsilon/\Delta s = (\varepsilon_{max} - \varepsilon_{min})/(s_{max} - s_{min})$$

where

ε_{max} and ε_{min} are the upper and lower residuals, respectively;

s_{max} and s_{min} are the upper and lower signal values, respectively.

Typical range for the ratio $\Delta\varepsilon/\Delta s$ is 0,01 to 0,02.

Alternatively, a one-way analysis of variance can be carried out to confirm the non-linearity.

If the ratios between concentrations and instrument readings are not strictly linear over the whole range of measurements, adjust the linearity of the instrument response for the component, in accordance with the manufacturer's instructions.

NOTE Milk from animals other than cows may have higher concentrations of fat and protein. For such milks better performance may be obtained if the linearity is adjusted and checked specifically for the relevant concentration range.

6.1.2 Purging efficiency of the cell

After a single pumping sequence of a sample through the instrument cell, the residual volume of the previous samples shall not exceed 1 % of the total volume of the cell.

To check the effectiveness of rinsing, analyse 20 consecutive samples of water and homogenized whole milk, using the sequence: water, water, milk, milk, water, water, etc., and record for each sample of water and milk the readings at all wavelengths used. Calculate for each wavelength the purging efficiency, E, using the formula:

$$E = (\Sigma M_1 - \Sigma W_2)100/(\Sigma M_2 - \Sigma W_2)$$

where

M_1 is the first reading for milk;

M_2 is the second reading for milk;

W_2 is the second reading for water at the same wavelength.

The value of this ratio shall not be less than 99 %.

6.1.3 Homogenization

6.1.3.1 To check the efficiency of the homogenizer, make two consecutive analyses, first with an unhomogenized whole milk sample, and secondly with the same whole milk sample after it has been homogenized through the instrument's homogenizer. The difference between the two fat readings shall not exceed 0,05 % for a milk sample containing a mass fraction of 3,5 % milkfat. To calculate the appropriate pass/fail criteria for milkfat concentrations other than 3,5 %, multiply the actual fat content by 0,014 3 to obtain the new criteria.

NOTE This procedure is not applicable to some instruments.

CAUTION: The results of this test can be misleading, as an instrument in which the homogenizer does not work at all will give very little difference between the first and the second run. A safer but more laborious alternative method is described in 6.1.3.2 (see reference [2]).

6.1.3.2 Alternatively, 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. This can be verified by particle size analysis of the homogenized milk. An acceptable range for the diameter, d, of the fat globules in the reference homogenized milk is 0,75 µm to 0,85 µm, with a d(0,9) of 1,4 µm to 1,5 µm [d(0,9) means that 90 % of the milkfat has fat globules with a diameter of less than d]. At this particle size distribution, light scattering will be minimal at both the wavelengths corresponding to the determination of fat by filter A and by filter B.

6.1.4 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, preferably at regular intervals determined by trials. A once-a-week change, possibly before the week-end to allow time for the instrument to dry, is considered good practice.

6.2 Physicochemical and biological factors

6.2.1 Milk composition

The signal obtained at each wavelength is the result of the specific absorption by the component being determined and, to a greater or lesser extent, by the variations in concentration of the other major components, water included, and by salts.

The influence of the variations of the fat, protein and lactose content of milk is corrected by cross-correction or interaction factors that are specific to each wavelength and each type of instrument.

These interaction factors are calculated either by the manufacturer or by the user and are automatically introduced into the measurements.

The measurements to be carried out and the corresponding corrections that shall be applied are the following:

— no determination of the protein content at 6,5 µm without a simultaneous determination of the fat content and a correction of the protein reading for the fat content;

— no determination of the fat content at 3,5 µm without a simultaneous determination of the protein and lactose contents and a correction of the fat reading for the protein and lactose contents.

Although the adoption of other internal corrections is not considered compulsory, they are highly recommended because they significantly improve the accuracy of the measurements.

Every 3 months, check the correction factors of the instrument using, for instance, the methods described in annex B. The apparent interactions should be as close as possible to zero and should not exceed limits of ±0,02. Beyond these limits the cross-corrections shall be adjusted according to the manufacturer's recommendations.

The correction factors should be checked whenever any major part of the instrument, for instance the interference filters, is serviced or changed.

6.2.2 Fat

6.2.2.1 Fatty acid composition

The variations in the fatty acid composition of milk (mean molecular mass and degree of unsaturation) influence significantly the relationship between the results of the reference method and the infrared measurements at 5,7 µm and, to a lesser extent, at 3,5 µm.

When compositional variations occur throughout an entire population of milks (for example, seasonal variation, regional differences, or different species), it may be necessary to modify the calibration of the instrument.

6.2.2.2 Lipolysis

The liberation of fatty acids by the action of lipase changes the instrument's readings. An increase in the lipolysis index of 1 milliequivalent per 100 g of fat, as measured by the BDI method, changes the instrument's signal for fat by –0,022 % at 5,7 μm (Filter A), and by +0,006 % at 3,5 μm (Filter B), for a sample containing a mass fraction of fat of 3,5 %.

6.2.2.3 High fat content

When analysing milk samples with a mass fraction of fat higher than 7,0 %, poor repeatability and deviation from the standard curve (see clause 7) may occur. Check with the manufacturer that the instrument is equipped with a homogenizer suitable for this type of milk.

6.2.2.4 Physical condition of milkfat

If part of the milkfat appears on the surface in an oiled-off condition, the test sample pumped by the instrument will not be representative of the fat content of the sample. Oiled-off samples shall therefore be avoided. Care should be taken to re-incorporate cream layers sticking to the walls of vessels and caps.

6.2.3 Protein

6.2.3.1 Variation in non-protein-nitrogen (NPN)

The IR protein determination is based on absorption of infrared energy by the peptide bonds of the protein molecules, whereas the components of the NPN fraction hardly contribute to the instrument signal at the wavelengths where protein is measured. An instrument can be calibrated to produce a protein-nitrogen-based (see ISO 8968-5) or a total-nitrogen-based protein estimate (see ISO 8968-1 or ISO 8968-2) measured by the Kjeldahl method.

When the instrument operator makes the choice to use a protein calibration based on total nitrogen, he/she assumes that the NPN content of the milk samples used to calibrate the instrument is constant from sample to sample within each calibration set and from set to set. If the NPN varies from sample to sample within the calibration set, distortions of the slope adjustment of the corrected signal on the protein channel will cause a larger standard deviation of difference between the Kjeldahl total nitrogen (TN) reference method and the instrument results.

Variation in NPN content within and between sets of calibration milks used in different laboratories will increase the mean difference and the standard deviation of difference between instrument protein results in different laboratories when the calibration is based on TN. When TN is used as a calibration reference, it is important that the average NPN content of the milks used for calibration be as close to the population mean as possible and that the variation in NPN/TN ratio from sample to sample within the set be as small as possible. Analysts need to be aware of this source of error in protein calibration.

6.2.3.2 Variation in citric acid

Citric acid absorbs energy at 6,5 μm, i.e. where protein is also determined. Variation in citric acid content will consequently need to be compensated for by modification of the protein calibrations

6.2.3.3 Lipolysis

An increase in the lipolysis index of 1 milliequivalent per 100 g of fat, as measured by the BDI method, changes the instrument’s signal for protein by +0,013 % at 6,5 μm, for a test sample containing a mass fraction of protein of 3,0 %.

6.2.4 Preservatives

Preservatives may influence the IR response as well as the reference methods. These effects can be different for different components and may vary between individual instruments. It is therefore important that these specific effects be examined before implementing any kind of sample preservation in a calibration scheme.

7 Calibration of the instrument

7.1 Objective

It is desirable to adjust the instrument's signal at each wavelength, so that for each level of concentration of the component being measured the instrument reading is closely approximate to the value given by the reference method.

Internationally accepted reference methods for the determination of fat, protein and lactose shall be used; i.e. ISO 1211, parts 1, 2, 4 and 5 of ISO 8968, and parts 1 and 2 of ISO 5765, respectively. For practical reasons or under certain circumstances alternative methods, for example the Gerber butyrometric method for fat content and Amido Black method for protein content, can be used provided they are regularly checked with the corresponding reference methods.

Because infrared instruments have different calibration systems, no specific procedure can be given. The manufacturer shall supply the laboratories with the means to adjust the instrument to comply with the requirements given in 7.2.

7.2 Checking the initial calibration for fat, protein and lactose

7.2.1 Milk samples

Collect a certain number of herd samples representative of the total population of herds within the laboratory's area, and whose composition varies regularly over the entire range of concentration of each component being measured, that is, between a mass fraction of fat of about 2,5 % and 5,0 % and of protein of about 2,5 % and 4,0 %. For the measurement of samples from individual cows, the mass fraction of fat and protein should reach about 7,0 % and 5,0 % respectively. Normally the number of such samples should exceed 15 and is seldom more than 50.

If necessary, a preservative normally used by the laboratory may be added to the test sample. The sample should show no sign of physical deterioration; samples containing more than 10^6 somatic cells per millilitre should be discarded.

NOTE Representative samples are a set of calibration samples, representative of the target population, accounting for all known and unknown biological and environmental phenomena that influence the instruments responses for fat, protein and lactose concentration. The list of known phenomena forms the basis of the designed controlled part of the sample set. Randomly selected samples model the unknown phenomena; they form the natural part of the sample set.

7.2.2 Analyses

Analyse the individual samples in duplicate using the reference methods to give the results y_i and, in triplicate using the instrument which is being calibrated to give the results x_i.

7.2.3 Calculations

Calculate the arithmetic means x and y of the replicate for each individual sample and plot the values obtained (x and y) on a graph to check that no outliers are present; if necessary repeat the analyses.

For each component, determine the regression equation: $y = bx + a$ and the residual standard deviation (s_{yx}) from the regression. The value s_{yx} should not exceed 0,06 % for each component.

Then, calibrate the instrument in accordance with the manufacturer's instructions.

This standardization procedure is assumed to give a very high level of confidence in the calibration of the instrument, but at a relatively high cost. The following simpler methods are possible, however, with some chance of a less accurate calibration.

a) A central laboratory may carry out the calibration using a few reference milk samples whose composition has been obtained from a master instrument standardized at the central laboratory according to the specified procedure. These “transfer” calibration samples should cover the whole range of variation of fat, protein and lactose contents, with preferably no correlation between components. Samples can be prepared according to the procedure described in annex C.

b) Instead of analysing each individual herd sample, these may be combined into six to eight samples at different concentration levels.

c) From a representative bulk milk sample, 10 to 12 samples may be prepared at different concentration levels by adjusting the fat and protein contents with various proportions of skim milk, cream, retentate, and ultrafiltrate (see annex C).

7.3 Maintaining calibration and confirmation of calibration validity

Since many different types of instrument calibrations are used, by preference, choice or necessity, it is important to confirm the accuracy of the calibration with reference chemical tests on randomly selected actual samples the instrument is routinely expected to test.

Every week, or more frequently when the animal feeding practice changes, collect a small number (for example, 4 or 5) of representative bulk milk samples and carry out determinations by the reference method and by the instrument for each component.

If the mean of the algebraic differences between instrumental results and reference results is higher than the expected accuracy of the instrument (see 12.3), re-adjust the calibration. This is the only way to confirm that the calibration which was applied to the instrument with calibration samples of a selected kind, is truly reproduced as expected, with results obtained on the routinely tested samples.

Every 3 months check the calibration of the instrument using the method described in 7.2. The calibration should be checked whenever any major part of the instrument (cell, homogenizer, interference filters) is serviced or changed.

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

9 Uniformity of test samples

To verify the uniformity of test portions, prepare them for calibration check, ring test or control (pilot) milk samples in the following way. Select at random 5 % of test portions from the same test sample. Measure the test portions as a series of single determinations on an IR instrument. Calculate the standard deviation on the fat results. If this is below 0,02, the uniformity is acceptable.

10 Determination

Follow the instructions given by the manufacturer for the measurement of fat, protein and lactose content of milk samples. Prior to analysis (homogenization), the test sample should be heated to 40 °C ± 1 °C and mixed thoroughly by inversion.