

Milk products — Guidelines for the application of near infrared spectrometry

ICS 67.100.01

National foreword

This British Standard was published by BSI. It is the UK implementation of ISO 21543:2006.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on AW/5 can be obtained on request to its secretary.

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 November 2006

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ISBN 0 580 49764 X

Amendments issued since publication

Amd. No.	Date	Comments

INTERNATIONAL
STANDARD

**ISO
21543**

**IDF
201**

First edition
2006-09-01

**Milk products — Guidelines for the
application of near infrared spectrometry**

*Produits laitiers — Lignes directrices pour l'application de la
spectrométrie dans le proche infrarouge*



Reference numbers
ISO 21543:2006(E)
IDF 201:2006(E)
Documents
Document Sharing Hub

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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 2.

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.

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.

ISO 21543|IDF 201 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.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has 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.

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 21543|IDF 201 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 Joint ISO-IDF Action Team on *Automated methods*, of the Standing Committee on *Quality assurance, statistics of analytical data and sampling*, under the aegis of its project leader, Mr L.K. Sørensen (DK).

Milk products — Guidelines for the application of near infrared spectrometry

1 Scope

This International Standard provides guidance on use of near infrared spectrometry in the determination of

- the total solids, fat and protein contents in cheese,
- the moisture, fat, protein and lactose contents in dried milk, dried whey and dried butter milk, and
- the moisture, fat, non-fat solids and salt contents in butter.

2 Terms and definitions

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

2.1

near infrared instrument

NIR instrument

proprietary apparatus which, when used under the conditions defined in this International Standard, estimates the mass fractions of the substances specified in Clause 1

2.2

total solids, moisture, non-fat solids, fat, protein, lactose and salt contents

mass fraction of substances determined using the method specified in this International Standard

NOTE These contents are expressed as mass fractions in percent.

3 Principle

The sample is pretreated to obtain a homogeneous test sample representing the chemical composition of the sample material. It is loaded into the sample holder of the NIR spectrometer. The absorbance at wavelengths in the near infrared region is measured and the spectral data are transformed to constituent concentrations by calibration models developed on representative samples from the population to be tested.

4 Reagents

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

4.1 Ethanol, or other appropriate solvent or detergent mixture, for cleaning re-usable sample cups.

5 Apparatus

5.1 Near-infrared (NIR) instrument, based on diffuse reflectance or transmittance measurement in the whole near infrared wavelength region of 700 nm to 2 500 nm or segments of this or at selected wavelengths.

The optical operation principle may be dispersive (e.g. grating monochromators), interferometric or non-thermal (e.g. light-emitting diodes, laser diodes and lasers). The instrument should be provided with a diagnostic test system for testing photometric instrument noise, wavelength accuracy and wavelength precision (for scanning spectrophotometers). The wavelength accuracy should be better than 0,5 nm and the repeatability standard deviation better than 0,02 nm.

The instrument should be equipped with a sample holder, which allows measurement of a sufficiently large sample volume or surface to eliminate any significant influence of inhomogeneity derived from the chemical composition or physical properties of the test sample. The sample path length (sample thickness) in transmittance measurements should be optimized according to the manufacturer's recommendations with respect to signal intensity for obtaining linearity and maximum signal/noise ratio. In reflectance measurements, a quartz window or other appropriate material to eliminate drying effects should preferably cover the interacting sample surface layer.

The sample cup (cuvette) may be re-usable or made of disposable material.

5.2 Grinding or grating device, appropriate for preparing the sample (e.g. a food processor for semi-hard cheese).

Changes in grinding or grating conditions may influence the NIR measurements.

6 Calibration and initial validation

6.1 Selection of calibration samples

The instrument should be calibrated before being used. Because of the complex nature of near infrared spectral data, which are mainly overtones and combination bands of fundamental vibrations in the mid-infrared region, the instrument should be calibrated using a series of natural samples (often at least 120 samples).

The accuracy and robustness of calibration models are dependent on the strategies used for sample selection and calibration. Developed calibration models are only valid for samples covered by the domain of the calibration samples. The first step in calibration development is therefore to define the application (e.g. sample types and concentration ranges). When calibration samples are selected, care should be taken to ensure that all major factors affecting the accuracy of calibration are covered within the limits of the defined application area. These factors include the following:

- a) combinations and composition ranges of major and minor sample components: analytes (e.g. total solids, fat and protein) and non-analytes;
- b) seasonal, geographic and genetic effects on milk composition;
- c) processing techniques and conditions;
- d) ripening stages of cheeses;
- e) storage and storage conditions.

The accuracy of calibration is influenced by the extent of variation in the sample material and the analyte concentration range. A moderate variation is usually easier to fit than a large variation. If the required accuracy cannot be obtained by a single calibration, then the application area should be split up into static or dynamic sub-areas, each with an associated calibration, in order to fulfil the requirements. Dynamic sub-areas are used in locally weighted regression algorithms where calibration samples close in spectral space to the actual prediction sample are selected from a larger population to create a local calibration equation.

It is generally preferable that the whole calibration range be covered in a uniform way, with samples from low to high concentrations of analytes. The sample spread should also be as uniform as possible with respect to the other variables, including those mentioned above. Furthermore, the samples should be collected and measured over a certain period of time to ensure inclusion of time-dependent effects. This design will improve the ruggedness and give a more even performance of the calibration over the entire analyte concentration range.

Multivariate methods [1], [2] may be used as a tool in the selection of samples to ensure a homogeneous calibration set covering all variation in spectroscopic data induced by chemical, biological and physical factors without duplication of samples with similar information. In practice, a larger sample population is measured by NIR spectroscopy for collection of NIR data only. Then samples differing in spectral information are selected for reference analyses. Identification of differing samples may be obtained from inspection of score plots from principal component analysis (PCA) using, for example, the first three components. This may be less practical in the case of many samples. However, it is recommended always to perform a PCA and inspect score plots to obtain a visual overview of the sample set. More formal cluster analyses may be obtained using techniques based on distance measurements [2]. Further samples may be added over a period of time to this pool of selected samples using PCA space or distance measurement to identify differing samples.

6.2 Reference analyses and NIR measurements

Internationally accepted reference methods for the determination of analytes should be used. The reference method used for calibration should be in statistical control; i.e. the variability should consist of a constant system of random variations. To support assessment of outliers, it may be useful to perform replicate analyses in independent series (different analysts, different equipment, etc.).

All major variations in NIR measuring conditions that may appear in practice should be built into the calibration model. An important factor is sample temperature.

The sampling procedure used and the sample size measured by NIR spectroscopy may be critical for the accuracy obtained [3]. The test sample volume or surface interacting in measurements should be large enough to avoid sample inhomogeneity having a significant influence. Reflectance measurement at higher wavelengths normally requires a larger sample surface than transmittance measurement at shorter wavelengths because the light penetration is much less. The optimal sample size should be determined from experiments where the prepared sample material (see 9.1) is measured repeatedly after repacking of the sample cup.

Special care should be taken to avoid surface drying effects, particularly in reflectance measurements.

The NIR measurements and reference analyses should preferably be performed on the same test sample in order to eliminate effects related to sampling uncertainty. The NIR measurements and the initiation of reference analyses should also be performed with a minimum time lag (preferably less than one day). It is good practice to randomize the order in which the samples are presented for both the reference analysis and NIR measurement.

6.3 Calibration

Because NIR instruments apply different calibration systems, no specific procedure can be given for calibration. However, the person performing the calibration should be familiar with the statistical principles behind the calibration algorithm used.