INTERNATIONAL STANDARD

ISO 8292-1

First edition
2008-04-01

Animal and vegetable fats and oils — Determination of solid fat content by pulsed NMR —

Part 1: Direct method

Corps gras d'origines animale et végétale — Détermination de la teneur en corps gras solides par RMN pulsée —
Partie 1: Méthode directe

Reference number
ISO 8292-1:2008(E)

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Published in Switzerland

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Foreword

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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 8292-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This part of ISO 8292, together with ISO 8292-2, cancel and replace ISO 8292:1991.

ISO 8292 consists of the following parts, under the general title *Animal and vegetable fats and oils — Determination of solid fat content by pulsed NMR*:

— *Part 1: Direct method*

— *Part 2: Indirect method*

Animal and vegetable fats and oils — Determination of solid fat content by pulsed NMR —

Part 1: Direct method

1 Scope

This part of ISO 8292 specifies a direct method for the determination of solid fat content in animal and vegetable fats and oils (hereafter designated “fats”) using low-resolution pulsed nuclear magnetic resonance (NMR) spectrometry.

Two alternative thermal pre-treatments are specified: one for general purpose fats not exhibiting pronounced polymorphism and which stabilize mainly in the β′-polymorph; and one for fats similar to cocoa butter which exhibit pronounced polymorphism and stabilize in the β-polymorph. Additional thermal pre-treatments, which may be more suitable for specific purposes, are given in an informative annex.

The direct method is easy to carry out and is reproducible, but is not as accurate as the indirect method due to the approximate method of calculation.

NOTE An indirect method is specified in ISO 8292-2.

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 661, *Animal and vegetable fats and oils — Preparation of test sample*

ISO 8292-2, *Animal and vegetable fats and oils — Determination of solid fat content by pulsed NMR — Part 2: Indirect method*

3 Terms and definitions

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

3.1
solid fat content
SFC
ratio as a percentage of the number of protons in the solid phase to the number of protons in the solid and liquid phase at a specified temperature

NOTE SFC expressed on this basis is taken to be numerically equivalent to the percentage mass fraction of fat in the solid state. No correction is made for the different densities of protons in the solid and liquid phases, because this would require exact knowledge of the composition of the solid and liquid phases of the fat blends at each temperature. Regardless of any other systematic errors, this means that SFC values obtained by this method are about 0,5 % to 1,0 % higher than the true solid fat percentage mass fraction.

3.2
liquid fat content
percentage mass fraction of fat in the liquid state at a specified temperature

NOTE The liquid fat content is equal to $100 - w_{SFC}$, where w_{SFC} is the solid fat content.

3.3
tempering
thermal treatment of the fat, after crystallization and prior to equilibration at the measurement temperature, which consists of holding the fat at a specified temperature for a specified time to transform the fat to a desired polymorph, and/or to ensure that a desired phase equilibrium has been achieved and/or to ensure that crystallization is complete

3.4
measurement temperature
temperature at which the solid fat content is determined

3.5
repetition time
interval between successive pulses

3.6
dead time
time during which the instrument receiver is unable to record the decay signal

NOTE Dead time is usually less than 10 µs after the pulse.

3.7
measurement protocol
complete description of the solid fat content determination specifying application, instrumental conditions, method, tempering, and whether measurements are in series or in parallel

NOTE Measurement protocols are listed in Table 1 and Annex C.

4 Symbols and abbreviated terms

f	conversion (extrapolation) factor to correct the NMR signal observed at 11 µs to that at time zero
n_p	number of pulses
S_1	magnetization decay signal measured at about 11 µs
S_2	magnetization decay signal measured at about 70 µs
SFC	solid fat content
S_L	magnetization decay signal corresponding to the liquid phase
S_S	magnetization decay signal corresponding to the solid phase
S_{S+L}	magnetization decay signals corresponding to both solid plus liquid phases
t_{rep}	repetition time
$w_{SFC,i}$	"true" SFC (measured in accordance with ISO 8292-2)
$w_{SFC,T}$	SFC at measurement temperature, T

5 Principle

The sample is tempered to a stable state at a specific temperature and then heated to, and stabilized at, the measurement temperature. Unless otherwise specified, measurement temperatures can be any or all of: 0 °C; 5 °C; 10 °C; 15 °C; 20 °C; 25 °C; 27,5 °C; 30 °C; 32,5 °C; 35 °C; 37,5 °C; 40 °C; 45 °C; 50 °C; 55 °C; 60 °C.

After electromagnetic equilibration in the static magnetic field of the NMR spectrometer and application of a 90º radio frequency pulse, the magnetization decay signals from the protons in the solid and liquid phases are recorded at about 11 µs and about 70 µs (or at times recommended by the spectrometer manufacturer, see 6.1). SFC is then calculated.

Measurements may be made in series or in parallel.

One tube is filled from each test sample when making measurements in series. After tempering as required and holding at 0 °C, the measurement tube is moved to the first measurement temperature, held for the specified time, the SFC measured, and then moved to the second measurement temperature, and so on. Thus, only one tube is required for all test samples, regardless of how many measurement temperatures are used. However, the SFC recorded at a given measurement temperature depends on the preceding measurement temperatures and times.

As many measurement tubes are filled from each test sample as there are measurement temperatures when making measurements in parallel. After tempering as required and holding at 0 °C, each measurement tube is moved more or less simultaneously to each required measurement temperature and held for the specified time before measuring the SFC.

Although more tubes are required for measurement in parallel than with that in series, each $w_{SFC,T}$ determination is independent of other determinations. Additionally, the total time for the measurements is significantly shortened.

EXAMPLE For a holding time of 90 min at 0 °C and holding times of 60 min at measurement temperatures of 10 °C, 20 °C, 30 °C, and 40 °C, the series measurement would take 5,5 h, whereas the parallel measurement would take 2,5 h.

6 Apparatus

6.1 Pulsed nuclear magnetic resonance spectrometer, low resolution

The NMR spectrometer shall have:

a) a magnet with a sufficiently uniform field to ensure that the half-life of the magnetization of a reference sample of liquid fat is longer than 1 000 µs;

b) a measurement dead time plus pulse width of less than 10 µs;

c) an automatic measuring device which operates as soon as the measurement tubes (6.2.1) are inserted;

d) an adjustable measurement repetition time;

e) a 10 mm measurement cell/probe for measurement tubes which is temperature controlled at 40 °C.

For exact magnetization decay signal times, refer to spectrometer manufacturer's instructions; these are normally at about 11 µs and about 70 µs and should not need to be altered by the user.

For preference, the instrument should be equipped with a computer which automatically takes the required measurements, performs the required calculations and presents the results directly on the computer screen or other display.

6.2 Tubes

6.2.1 Measurement tubes, of glass with plastic caps, with outer diameter (10 ± 0,25) mm, wall thickness (0,9 ± 0,25) mm, and length at least 150 mm, or as specified by the NMR spectrometer manufacturer.

6.2.2 Calibration tubes, of known instrument response to calibrate the spectrometer and to check the direct method.

NOTE Plastic-in-oil calibration materials with known responses, giving an *f* factor in the range 1,4 to 1,45 appropriate for the instrument and for use with the non-stabilizing direct and other protocols (see Table 1 and Annex C) are supplied by the instrument manufacturer in standard measurement tubes. Materials giving SFC mass fractions of 0 %, about 30 % and about 70 % are suitable. These values are independent of temperature. The calibration tubes need re-calibration at intervals as specified by the supplier.[1)]

6.3 Temperature-maintenance equipment

6.3.1 General

In principle, temperature-controlled blocks (6.3.3) have advantages over water baths (6.3.2) because the tubes can never come into contact with water. In practice, as with aluminium blocks in water baths, the tubes can take a significant time to come to the set temperature. Heat transfer can be improved if the tube wells are purged with a dry gas. Blocks are also more difficult to control precisely than water baths, although modern electronic controls can provide the required precision.

6.3.2 Water baths

Baths are required at temperatures of (0 ± 0,1) °C, (60 ± 0,1) °C, and, to within ± 0,1 ºC, the measuring and tempering temperatures required according to the measurement protocol chosen. For the 60 °C, measurement temperature, and tempering temperature baths, temperature-controlled blocks (6.3.3) may be substituted.

Each water bath shall be equipped with either one aluminium block (6.3.2.1) or one metal rack (6.3.2.2) to accommodate measurement tubes (6.2.1) immersible in the water to a depth of 60 mm.

Metal racks are preferred to aluminium blocks, especially when a large number of test samples with high SFC are being measured or when the rapid or ultra-rapid measurement protocols are being used. When using aluminium blocks, there may be a significant time lag after the tube is inserted before the fat in the tube reaches the set temperature of the water bath. The perceived advantage of blocks is that the tubes can remain dry and do not need to be wiped dry with a paper tissue before insertion into the spectrometer. In practice, however, it is usually found that due to splashing or condensation, the tubes do become wet so that drying is always recommended, see 8.9.

6.3.2.1 Aluminium blocks, with holes of diameter (10,35 ± 0,1) mm, and depth 70 mm. The thickness of the metal under the holes and the distance between the edge of a peripheral hole and the nearest side face shall be 10 mm. The distance between the axes of two adjacent holes shall be at least 17 mm (centre to centre).

6.3.2.2 Metal racks, open-sided, with holes of diameter 11 mm to 15 mm; the distance between the axes of two adjacent holes shall be at least 20 mm (centre to centre).

6.3.3 Temperature-controlled blocks, with holes

The blocks, with electronic control, shall be capable of being maintained to within ± 0,1 ºC of a set temperature. These blocks may be used instead of water baths [except the 0 °C bath (6.3.2), because of the large amount of cooling required]. The diameter of the holes shall be (10,35 ± 0,1) mm.

1) It is expected that in the future “open and independent” standards will be available from the EU’s Institute for Reference Materials and Measurements in Geel, Belgium. This information is given for the convenience of users of this International Standard, and does not constitute an endorsement of these products by ISO.

Blocks are particularly useful at temperatures of 35 °C or more when no cooling is required (assuming the ambient room temperature is below 22 °C) and where temperature control is less critical because of the usually lower absolute solid fat levels.

6.4 Oven, with fan

The oven shall be capable of being maintained at (80 ± 2) °C.

Since the purpose of the 80 °C temperature is to melt the test portion and destroy its previous thermal history, it shall be at least 20 °C above the melting temperature of the fat. If this is not the case, then the oven temperature shall be raised accordingly and the fact recorded in the test report (Clause 11). This is rarely necessary, as the fats concerned contain large amounts of long-chain saturated fatty acids, e.g. fully hydrogenated liquid vegetable oils.

Although a water bath (6.3.2) or temperature-controlled block (6.3.3) may be used for the 80 °C temperature, it is preferable to use an oven. In a block or bath it is almost inevitable that fat will contact the sides, at a temperature above that of immersion, when filling the tubes. An oven ensures that all the fat in the tube is completely melted and there are no seed crystals remaining with an unknown thermal history which could seed the molten fat when it is eventually moved to the 0 °C crystallization temperature. Thus, an oven is likely to give more reliable and reproducible results.

6.5 Stop-clock

An analogue clock with a large sweep second hand is preferred, although a digital clock may be used.

7 Sampling

A representative sample shall have been sent to the laboratory. It shall not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 8292. A recommended sampling method is given in ISO 5555.

8 Procedure

8.1 Measurement protocol and test sample

Choose the required protocol from Table 1 according to the sample type and other requirements. For some types or applications of fats, the protocols given in Table 1 are not appropriate. The measurement protocols given in Annex C may be more suitable.

Prepare the test sample in accordance with ISO 661.

Table 1 — Measurement protocols

Measurement protocol		Applicable to	Instrumental conditions	First time at 0 °C	Tempering		Second time at 0 °C	Measurement conditions	
No.	Name				Time	Temp.		Type	Time
				min	h	°C	min		min
1D	Non-stabilizing direct	Fats and blends (comprising mainly vegetable fats, hydrogenated and/or interesterified) crystallizing in the β'-polymorph and as used for margarines, spreads, shortenings and other general food applications	f = 1,4 to 1,45; repetition time [a], t_{rep} = 2 s; No. pulses [b], n_p = 3	—	—		(60 ± 2)	Parallel	(30 ± 1)
2D	β-Stabilizing direct	Cocoa butter, cocoa butter equivalents and similar fats containing large amounts of 2-oleo-di-saturated triacylglycerols and crystallizing in the β-polymorph	f = 1,6 to 1,65; repetition time, t_{rep} = 6 s; No. pulses [c], n_p = 1	(90 ± 2)	(40 ± 0,5)	26	(90 ± 2)	Parallel	(60 ± 2)

[a] Needs to be 6 s for fats in the β-polymorph.

[b] Pulse data are averaged by the instrument. Ideally, three pulses are used, but some older instruments can be set to only either one or four (1^2 or 2^2) pulses, in which case use four pulses.

[c] Use of three pulses would result in sufficient time in the measurement cell to cause the test portion to partially melt and the SFC to reduce during the measurement.

8.2 Oven, water baths and temperature-controlled blocks

Set this equipment up for the required temperatures as specified in the protocol.

8.3 Determination of the conversion factor (where necessary)

Due to the dead time of the instrument, the first measurement can be made only after the signal from the solid phase has reduced significantly. A conversion factor corrects approximately for this effect.

Although the calibration tubes containing plastic-in-oil standards give a reproducible, but only approximately correct, conversion factor for the common β′-polymorphic general-purpose fats averaged over the temperature range of interest, they do not give the correct factor for the β-polymorphic fats such as cocoa butter. For these fats, and for any other fats or blends of fats for which the polymorphism is unknown, to avoid significant systematic errors, it is desirable to determine a better estimate of the conversion factor.

For the fats or fat blends of interest, set up to determine SFC in accordance with both ISO 8289-2, i.e. measure a liquid oil reference, as well as by this part of ISO 8292. Record the SFC as determined by this part of ISO 8292 in the usual way, but also record S_1 and S_2 for that measurement. (Consult the spectrometer manual for how to do this.)

For each test sample, calculate the "true" SFC, $w_{SFC,i}$, using ISO 8292-2.

For each test portion, work out the extrapolation factor, f, required to equate the indirect and direct SFC determinations, and given by Equation (1):

$$f = \frac{w_{SFC,i} \times S_2}{(100 - w_{SFC,i}) \times (S_1 - S_2)} \quad (1)$$

where

$w_{SFC,i}$ is the "true" SFC;

S_1 is the magnetization decay signal measured at about 11 µs;

S_2 is the magnetization decay signal measured at about 70 µs.

Calculated factors vary according to the blend/sample and the temperature. This is correct, particularly the temperature variation, which the direct method ignores. Work out an average which gives the best results. It is suggested that results in the 20 °C to 30 °C range be averaged, as this is where solids are likely to be nearest to 50 % mass fraction where the factor difference has most effect. For cocoa butter and similar fats which crystallize in a β-polymorph, the factor is in the range 1,6 to1,7.

Because of the impossibility of knowing what the true factor should be for many blends of β-polymorphic fats, such as cocoa butter, with β′-polymorphic fats, such as milk fat or palm fractions, it is recommended to use ISO 8292-2 for all such blends to determine the true SFC.

Should the results be measured using an incorrect factor, they can easily be recalculated using Equation (2):

$$w_{SFC}^{corr} = \frac{w_{SFC}^{err} f^{corr}}{f^{err}(100 - w_{SFC}^{err}) + w_{SFC}^{err} f^{corr}} \times 100 \quad (2)$$

where superscripts “err” and “corr” refer to erroneous and corrected values, respectively. For example, if the $w_{SFC,30}$ value of a cocoa butter test sample was measured as 49,0 % mass fraction using f = 1,41 (i.e. w_{SFC}^{err} = 49,0), but it is known that the correct value for the instrument is f = 1,64, then Equation (2) gives w_{SFC}^{corr} = 52,8 %.

Some variation in f between instruments at various sites is unavoidable, because f depends partly on the instrument. Therefore, during the establishment of commercial contracts, reference samples should be exchanged to agree on the solids content and the appropriate f to be used. For example, for measurement protocol 2D, it would be appropriate to exchange a standard reference cocoa butter sample to determine the correct f.

8.4 NMR spectrometer

8.4.1 Calibration

Using the calibration tubes (6.2.2), calibrate the spectrometer according to the manufacturer's instructions and at the intervals recommended by the manufacturer.

8.4.2 Instrumental conditions

Set the conditions for the spectrometer according to the measurement protocol chosen in 8.1.

8.4.3 Checking

Daily, or before each direct method determination, check the spectrometer as follows:

a) insert each of the three calibration tubes (6.2.2) into the spectrometer in turn and record the SFC;

b) repeat the measurements;

c) the measured SFC of each tube shall not deviate by more than 0,3 % absolute from the known, calibration, value.

If any SFC does deviate, then f shall be altered and the checking repeated until the three calibration tubes do not deviate by more than 0,3 %. Alternatively, it may be necessary to recalibrate the spectrometer (see 8.4.1).

8.5 Filling the measurement tubes

Fill the tubes with approximately 2 ml of fat or a depth of between 30 mm and 50 mm, or as specified by the instrument manufacturer. Cap the tubes and place in racks that keep the tubes vertical. If metal racks (6.3.2.2) are used, it is very convenient and time saving to put the filled tubes directly into the racks. The test portions can then be moved conveniently to the oven and to the water baths without further transfers and handling.

For measurements in parallel, fill one measurement tube from each test sample for each measurement temperature; for measurements in series, fill a single measurement tube sequentially from each test sample.

8.6 Removing the thermal history

When all the required tubes have been filled, transfer them to the oven (6.4). Hold at the oven temperature for a minimum of 15 min.

8.7 Equilibrating at the initial temperature

Transfer all the tubes to the 60 °C water bath (6.3.2) or block (6.3.3). Hold for a minimum of 15 min. The time may be longer than this, but shall not be shorter as otherwise complete equilibration may not be achieved.