INTERNATIONAL STANDARD

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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
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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 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 $w_{\mathrm{SFC},T}$ 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.

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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, $w_{\mathrm{SFC,i}}$, 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):

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

where

$w_{\mathrm{SFC,i}}$ 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):

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

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.

8.8 Crystallization and tempering

From this stage onwards, all the times shall be maintained within the tolerances specified here or in the measurement protocol.

If required by the chosen measurement protocol, transfer the tubes into the 0 °C bath. Leave in the 0 °C bath for the time specified in the “First time at 0 °C” column of Table 1 or Annex C.

If required by the chosen measurement protocol, transfer the tubes into the tempering bath set to the specified temperature. Leave in the tempering bath for the specified time.

At (1,0 ± 0,5) min or (2,0 ± 0,5) min intervals, transfer the tubes into the 0 °C bath (or liquid nitrogen for the ultra-rapid protocol). Leave in the 0 °C bath for the time specified in the “First time at 0 °C” column of Table 1 or Annex C. See 8.9 for choice of interval.

8.9 Measuring the SFC

In most circumstances and as given in Table 1, make the measurements in parallel.

NOTE Series measurement can occasionally be appropriate when only small amounts of test sample are available or less preparation time is needed. It is also appropriate, as in measurement protocol 4D (see Annex C) when the best comparability with previous, dilatometric, methods of determining solid content is required.

8.9.1 Measurement in parallel

At (1,0 ± 0,5) min or (2,0 ± 0,5) min intervals, transfer the tubes for each test portion to each of the measurement temperature water baths (6.3.2) or blocks (6.3.3). The number of tubes transferred at each 1 min or 2 min interval will be the same as the number of measurement temperatures. The choice of time interval will depend on the number of tubes/temperatures to be measured, the skill of the operator and the layout of the apparatus.

NOTE Experience shows that it is easily possible to transfer a tube from bath or block to the spectrometer and make the measurement within 15 s. Therefore, six tubes/measurement temperatures may comfortably be processed within 2 min.

After the time specified in the measurement protocol, in exactly the same sequence as they were placed in the measurement temperature baths or blocks, transfer the tubes to the spectrometer at the same (1,0 ± 0,5) min or (2,0 ± 0,5) min intervals. Wipe each tube briefly with a soft paper tissue to remove all water, before placing it in the measurement cell. Record the SFC reading. Record a reading of zero if the test portion is visibly completely clear.

8.9.2 Measurement in series

At (1,0 ± 0,5) min intervals, transfer the tube containing a test portion to the first (lowest) of the measurement temperature water baths (6.3.2) or blocks (6.3.3).

After the time specified in the measurement protocol, in exactly the same sequence as they were placed in the measurement temperature baths or blocks, transfer the tubes to the spectrometer. Wipe each tube briefly with a soft paper tissue to remove all water, before placing it in the measurement cell. Record the SFC.

Transfer the tubes containing each test portion to the second (next lowest) of the measurement temperature baths or blocks at (1,0 ± 0,5) min intervals.

Repeat the procedures from the second paragraph until all tubes have been measured.

If the NMR spectrometer is not equipped with a computer or other automatic calculation device, then record the signals manually and compute the SFC according Equation (3) (see Clause 9).

IMPORTANT — For reliable and reproducible results, adhere to the times and tolerances specified. This is easily achieved using a laboratory stop-clock (6.5), preferably an analogue clock with a large sweep second hand, moving the tubes as the clock moves round to the appropriate time. Alternatively, if a digital clock is used, it is convenient to set it to 0:00 or 12:00 at the start.

8.10 Number of determinations

Carry out one determination on each of two test portions in separate tubes taken from the same test sample.

8.11 Cleaning the measurement tubes

Measurement tubes shall be clean, dry, and free from all fat from previous measurements before filling with the test portion. Because of the narrow diameter of the tubes, cleaning often proves to be a problem. Solvents or narrow brushes are often used. Tubes may be cleaned in a laboratory automatic washer or a standard domestic dishwasher. However, for cleaning to be effective, it is necessary to ensure that the tubes are free of most of the fat and are maintained more or less vertically in the washer. This may be achieved as follows.

Either use a laboratory washer, equipped with special support “fingers” which can just fit into the tube and inject hot detergent solution inside.

Or use a washer without special “fingers” by supporting the tubes in a wire-mesh rack with slots of the correct size to take the tubes. The rack should be equipped with a wire-mesh lid to retain the tubes when the rack is inverted. An advantage of such a rack is that, as they are finished with at the end of the measurement sequence, the tubes can be placed directly upside down in the rack and then the filled rack placed in the 80 °C oven for some time to allow the fat to melt and most of it to drain away. Still inverted, the rack can then be transferred to the washer. After washing and drying, the tubes rack can be used as a convenient holder or the tubes removed and stored ready for reuse.

9 Expression of results

If the NMR spectrometer is not equipped with a computer or other automatic calculation device to read off the results, use the manually recorded signals to calculate the SFC at a given temperature, $w_{\mathrm{SFC},T}$, as a percentage mass fraction, using Equation (3):

$$w_{\mathrm{SFC},T} = \frac{f(S_1 - S_2)}{f(S_1 - S_2) + S_2} \times 100 \qquad (3)$$

where

f is the conversion (extrapolation) factor to correct the NMR signal observed at 11 µs to that at time zero;

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

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

See Annex B for more details of the theory.

Express the result as the arithmetic mean of the two determinations (8.10), provided that the requirement for repeatability (10.2) of each $w_{\mathrm{SFC},T}$ value is satisfied. Report the result to one decimal place.

10 Precision

10.1 Interlaboratory test

Details of the interlaboratory tests on the precision of the method are given in Annex A. The values derived from these tests may not be applicable to SFC ranges and fats other than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will in no more than 5 % of cases exceed the repeatability limit, r, given in or derived from Tables 2 and 3.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different materials, will in not more than 5 % of cases exceed the reproducibility limit, R, given in or derived from Tables 2 and 3.

Table 2 — Repeatability limit, r, and reproducibility limit, R, for measurement protocol 1D

Temperature	Repeatability limit, r			Reproducibility limit, R		
°C	Minimum	Maximum	Mean	Minimum	Maximum	Mean
10	0,30	1,29	0,76	1,82	6,85	4,02
20	0,21	1,47	0,72	1,46	4,87	2,51
25	0,39	0,89	0,55	1,60	5,95	3,11
30	0,24	1,79	0,74	0,35	11,50	3,28
35	0,22	0,68	0,51	0,20	2,77	1,95
40	0,07	0,96	0,48	0,18	1,99	1,10
Mean	**0,24**	**1,18**	**0,62**	**0,94**	**5,66**	**2,66**

Table 3 — Repeatability limit, r, and reproducibility limit, R, for measurement protocol 2D

Temperature	Repeatability limit, r			Reproducibility limit, R		
°C	Minimum	Maximum	Mean	Minimum	Maximum	Mean
20	0,13	1,15	0,71	1,22	4,76	3,40
25	0,30	0,88	0,60	1,68	5,65	3,78
30	0,61	2,71	1,29	3,13	10,95	7,16
35	0,43	2,61	1,57	0,93	10,41	3,48
40	0,00	1,09	0,53	0,17	2,53	1,02
Mean	**0,29**	**1,69**	**0,94**	**1,43**	**6,86**	**3,77**

NOTE Details of the fats used in the collaborative study are given in Annex A. Statistics are not given where only one test sample was measured at a temperature.

11 Test report

The test report shall specify at least the following information:

a) all information necessary for the complete identification of the sample;

b) details of the NMR spectrometer used;

c) the method used, with reference to this part of ISO 8292;

d) the measurement protocol used;

e) the measurement temperatures used;

f) whether a water bath with aluminium blocks, water bath with metal racks or temperature-controlled blocks were used for temperature control;

g) the results obtained;

h) all operating details not specified in this part of ISO 8292, or regarded as optional, together with details of any incidents which may have influenced the results.

Annex A
(informative)

Results of interlaboratory tests

Table A.1 — Summary of statistical evaluation — Measurement protocol 1D

	Hydrogenated soybean oil (A)	Shortening blend, hydrogenated hardstock (B)	Shortening blend, inter-esterified hardstock (C)	Palm kernel stearin (D)	Coconut oil (E)	Palm oil/palm stearin blend (F)
Slip melting point	~37 °C	~40 °C	~40 °C	—	—	~45 °C
Iodine value	—	—	—	~7	—	—
Determination temperature	10 °C					
No. participating laboratories	22	22	22	22	22	22
No. laboratories retained after eliminating outliers	21	22	22	21	22	20
No. test results, all labs	42	44	44	42	44	40
Mean	77,74	67,73	77,23	96,48	80,68	57,78
Repeatability standard deviation, s_r	0,23	0,30	0,28	0,11	0,46	0,24
Coefficient of variation of repeatability, CV(r)	0,3	0,4	0,4	0,1	0,6	0,4
Repeatability limit, $r = 2{,}8 s_r$	0,64	0,83	0,80	0,30	1,29	0,67
Reproducibility standard deviation, s_R	1,99	0,93	1,82	0,65	2,45	0,76
Coefficient of variation of reproducibility, CV(R)	2,6	1,4	2,4	0,7	3,0	1,3
Reproducibility limit, $R = 2{,}8 s_R$	5,58	2,60	5,10	1,82	6,85	2,14
Determination temperature	20 °C					
No. participating laboratories	23	23	23	23	23	23
No. laboratories retained after eliminating outliers	22	21	23	22	23	22
No. test results, all labs	44	42	46	44	46	44

Table A.1 (*continued*)

	Hydrogenated soybean oil (A)	Shortening blend, hydrogenated hardstock (B)	Shortening blend, inter-esterified hardstock (C)	Palm kernel stearin (D)	Coconut oil (E)	Palm oil/palm stearin blend (F)
Mean	56,49	43,51	54,14	94,58	39,16	35,28
Repeatability standard deviation, s_r	0,25	0,26	0,17	0,08	0,53	0,27
Coefficient of variation of repeatability, CV(r)	0,4	0,6	0,3	0,1	1,3	0,8
Repeatability limit, $r = 2{,}8 s_r$	0,70	0,72	0,49	0,21	1,47	0,75
Reproducibility standard deviation, s_R	1,02	0,69	0,74	0,65	1,74	0,52
Coefficient of variation of reproducibility, CV(R)	1,8	1,6	1,4	0,7	4,4	1,5
Reproducibility limit, $R = 2{,}8 s_R$	2,87	1,94	2,08	1,83	4,87	1,46
Determination temperature	25 °C					
No. participating laboratories	20	20	20	20	20	20
No. laboratories retained after eliminating outliers	19	16	16	19	16	17
No. test results, all labs	38	32	32	38	32	34
Mean	40,78	29,74	40,62	86,11	1,73	23,76
Repeatability standard deviation, s_r	0,21	0,15	0,14	0,32	0,21	0,14
Coefficient of variation of repeatability, CV(r)	0,5	0,5	0,3	0,4	12,3	0,6
Repeatability limit, $r = 2{,}8 s_r$	0,58	0,42	0,39	0,89	0,59	0,40
Reproducibility standard deviation, s_R	1,35	0,69	0,68	2,12	1,26	0,57

Table A.1 (*continued*)

	Hydrogenated soybean oil (A)	Shortening blend, hydrogenated hardstock (B)	Shortening blend, inter-esterified hardstock (C)	Palm kernel stearin (D)	Coconut oil (E)	Palm oil/palm stearin blend (F)
Coefficient of variation of reproducibility, CV(R)	3,3	2,3	1,7	2,5	72,9	2,4
Reproducibility limit, R = 2,8s_R	3,78	1,92	1,91	5,95	3,52	1,60
Determination temperature	30 °C					
No. participating laboratories	23	23	23	23	19	23
No. laboratories retained after eliminating outliers	21	21	21	21	19	21
No. test results, all labs	42	42	42	42	38	42
Mean	23,32	19,04	26,49	39,47	0,07	16,21
Repeatability standard deviation, s_r	0,20	0,28	0,21	0,64	0,09	0,17
Coefficient of variation of repeatability, CV(r)	0,8	1,5	0,8	1,6	116,6	1,0
Repeatability limit, r = 2,8s_r	0,55	0,77	0,59	1,79	0,24	0,47
Reproducibility standard deviation, s_R	0,95	0,56	0,87	4,11	0,13	0,43
Coefficient of variation of reproducibility, CV(R)	4,1	2,9	3,3	10,4	169,2	2,6
Reproducibility limit, R = 2,8s_R	2,66	1,56	2,43	11,50	0,35	1,19
Determination temperature	35 °C					
No. participating laboratories	23	23	23	23	18	23

Table A.1 (*continued*)

	Hydrogenated soybean oil (A)	Shortening blend, hydrogenated hardstock (B)	Shortening blend, inter-esterified hardstock (C)	Palm kernel stearin (D)	Coconut oil (E)	Palm oil/palm stearin blend (F)
No. laboratories retained after eliminating outliers	23	23	22	21	16	23
No. test results, all labs	46	46	44	42	32	46
Mean	9,49	11,43	15,02	2,77	0,03	11,52
Repeatability standard deviation, s_r	0,19	0,21	0,16	0,20	0,08	0,24
Coefficient of variation of repeatability, CV(r)	2,0	1,8	1,1	7,2	274,0	2,1
Repeatability limit, $r = 2{,}8 s_r$	0,53	0,58	0,46	0,56	0,22	0,68
Reproducibility standard deviation, s_R	0,99	0,79	0,97	0,74	0,07	0,61
Coefficient of variation of reproducibility, CV(R)	10,4	6,9	6,5	26,6	258,6	5,3
Reproducibility limit, $R = 2{,}8 s_R$	2,77	2,20	2,72	2,06	0,20	1,72
Determination temperature	40 °C					
No. participating laboratories	22	22	22	20	18	22
No. laboratories retained after eliminating outliers	22	22	22	18	15	21
No. test results, all labs	44	44	44	36	30	42
Mean	1,34	4,16	5,03	0,04	0,03	7,63
Repeatability standard deviation, s_r	0,23	0,19	0,34	0,06	0,03	0,19
Coefficient of variation of repeatability, CV(r)	17,1	4,5	6,8	160,6	96,8	2,5

Table A.1 (*continued*)

	Hydrogenated soybean oil (A)	Shortening blend, hydrogenated hardstock (B)	Shortening blend, inter-esterified hardstock (C)	Palm kernel stearin (D)	Coconut oil (E)	Palm oil/palm stearin blend (F)
Repeatability limit, r = 2,8s_r	0,64	0,52	0,96	0,16	0,07	0,53
Reproducibility standard deviation, s_R	0,48	0,53	0,71	0,06	0,09	0,48
Coefficient of variation of reproducibility, CV(R)	36,1	12,8	14,1	174,0	345,7	6,3
Reproducibility limit, R = 2,8s_R	1,35	1,50	1,99	0,18	0,26	1,34
Determination temperature	45 °C					
No. participating laboratories						10
No. laboratories retained after eliminating outliers						10
No. test results, all labs						20
Mean						3,88
Repeatability standard deviation, s_r						0,17
Coefficient of variation of repeatability, CV(r)						4,4
Repeatability limit, r = 2,8s_r						0,47
Reproducibility standard deviation, s_R						0,74
Coefficient of variation of reproducibility, CV(R)						19,2
Reproducibility limit, R = 2,8s_R						2,08

Table A.2 — Summary of statistical evaluation — Measurement protocol 2D

	Palm oil/palm stearin blend (F)	Cocoa butter, soft Brazilian type (G)	Cocoa butter, standard West African type (H)	Illipe butter (Borneo tallow, Tengkawang fat) (I)	Cocoa butter equivalent, standard type, $w_{SFC,30} \approx$ 35 % to 40 % (J)	Palm mid-fraction, hard/CBE grade, (K)
Slip melting point	~45 °C	—	—	—	—	—
Iodine value	—	—	—	—	—	~34
Determination temperature	10 °C					
No. participating laboratories	9					
No. laboratories retained after eliminating outliers	8					
No. test results, all labs	16					
Mean	54,84					
Repeatability standard deviation, s_r	0,37					
Coefficient of variation of repeatability, CV(r)	0,7					
Repeatability limit, r = 2,8 s_r	1,04					
Reproducibility standard deviation, s_R	2,37					
Coefficient of variation of reproducibility, CV(R)	4,3					
Reproducibility limit, R = 2,8 s_R	6,63					
Determination temperature	20 °C					
No. participating laboratories	9	10	10	10	10	10
No. laboratories retained after eliminating outliers	9	10	10	9	9	9
No. test results, all labs	18	20	20	18	18	18

Table A.2 (*continued*)

	Palm oil/palm stearin blend (F)	**Cocoa butter, soft Brazilian type (G)**	**Cocoa butter, standard West African type (H)**	**Illipe butter (Borneo tallow, Tengkawang fat) (I)**	**Cocoa butter equivalent, standard type,** $w_{SFC,30} \approx$ 35 % to 40 % (**J**)	**Palm mid-fraction, hard/CBE grade, (K)**
Mean	27,37	77,75	78,09	91,15	71,03	83,18
Repeatability standard deviation, s_r	0,27	0,40	0,41	0,05	0,15	0,24
Coefficient of variation of repeatability, CV(r)	1,0	0,5	0,5	0,1	0,2	0,3
Repeatability limit, r = 2,8 s_r	0,75	1,12	1,15	0,13	0,43	0,67
Reproducibility standard deviation, s_R	1,20	1,69	1,31	0,44	1,70	0,96
Coefficient of variation of reproducibility, CV(R)	4,4	2,2	1,7	0,5	2,4	1,2
Reproducibility limit, R = 2,8 s_R	3,36	4,72	3,67	1,22	4,76	2,69
Determination temperature	25 °C					
No. participating laboratories	10	10	10	10	10	10
No. laboratories retained after eliminating outliers	9	9	9	8	9	9
No. test results, all labs	18	18	18	16	18	18
Mean	24,48	74,69	72,24	89,24	55,58	70,41
Repeatability standard deviation, s_r	0,21	0,27	0,14	0,11	0,25	0,31
Coefficient of variation of repeatability, CV(r)	0,9	0,4	0,2	0,1	0,4	0,4
Repeatability limit, r = 2,8 s_r	0,60	0,76	0,38	0,30	0,69	0,88
Reproducibility standard deviation, s_R	1,13	1,57	1,37	0,60	2,02	1,42

Table A.2 (*continued*)

	Palm oil/palm stearin blend (F)	Cocoa butter, soft Brazilian type (G)	Cocoa butter, standard West African type (H)	Illipe butter (Borneo tallow, Tengkawang fat) (I)	Cocoa butter equivalent, standard type, $w_{SFC,30} \approx$ 35 % to 40 % (J)	Palm mid-fraction, hard/CBE grade, (K)
Coefficient of variation of reproducibility, CV(R)	4,6	2,2	1,9	0,7	3,6	2,0
Reproducibility limit, R = 2,8 s_R	3,15	4,39	3,84	1,68	5,65	3,97
Determination temperature	30 °C					
No. participating laboratories	10	10	10	10	10	10
No. laboratories retained after eliminating outliers	10	10	9	10	10	10
No. test results, all labs	20	20	18	20	20	20
Mean	21,47	48,09	51,52	81,76	31,04	43,73
Repeatability standard deviation, s_r	0,37	0,97	0,39	0,37	0,22	0,45
Coefficient of variation of repeatability, CV(r)	1,7	2,0	0,8	0,5	0,7	1,0
Repeatability limit, r = 2,8 s_r	1,03	2,71	1,09	1,04	0,61	1,27
Reproducibility standard deviation, s_R	1,12	3,16	2,78	1,45	2,93	3,91
Coefficient of variation of reproducibility, CV(R)	5,2	6,6	5,4	1,8	9,4	9,0
Reproducibility limit, R = 2,8 s_R	3,13	8,84	7,78	4,05	8,20	10,95
Determination temperature	35 °C					
No. participating laboratories	10	9	10	10	10	10
No. laboratories retained after eliminating outliers	10	8	10	10	10	10

Table A.2 (*continued*)

	Palm oil/palm stearin blend (F)	**Cocoa butter, soft Brazilian type (G)**	**Cocoa butter, standard West African type (H)**	**Illipe butter (Borneo tallow, Tengkawang fat) (I)**	**Cocoa butter equivalent, standard type,** $w_{SFC,30} \approx$ 35 % to 40 % (**J**)	**Palm mid-fraction, hard/CBE grade, (K)**
No. test results, all labs	20	16	20	20	20	20
Mean	15,20	0,52	1,52	31,69	1,73	4,81
Repeatability standard deviation, s_r	0,28	0,15	0,64	0,93	0,53	0,85
Coefficient of variation of repeatability, CV(r)	1,9	30,0	41,9	2,9	30,5	17,6
Repeatability limit, r = 2,8 s_r	0,79	0,43	1,76	2,61	1,48	2,37
Reproducibility standard deviation, s_R	1,08	0,33	0,80	3,72	0,57	0,95
Coefficient of variation of reproducibility, CV(R)	7,1	64,3	52,8	11,7	32,7	19,8
Reproducibility limit, R = 2,8 s_R	3,01	0,93	2,25	10,41	1,59	2,66
Determination temperature	40 °C					
No. participating laboratories	9	6	7	7	7	7
No. laboratories retained after eliminating outliers	9	6	7	7	6	5
No. test results, all labs	18	12	14	14	12	10
Mean	10,74	0,16	0,32	0,32	0,20	0,04
Repeatability standard deviation, s_r	0,38	0,12	0,13	0,39	0,12	0,00
Coefficient of variation of repeatability, CV(r)	3,6	77,5	39,5	123,1	59,3	0,0
Repeatability limit, r = 2,8 s_r	1,07	0,35	0,35	1,09	0,33	0,00

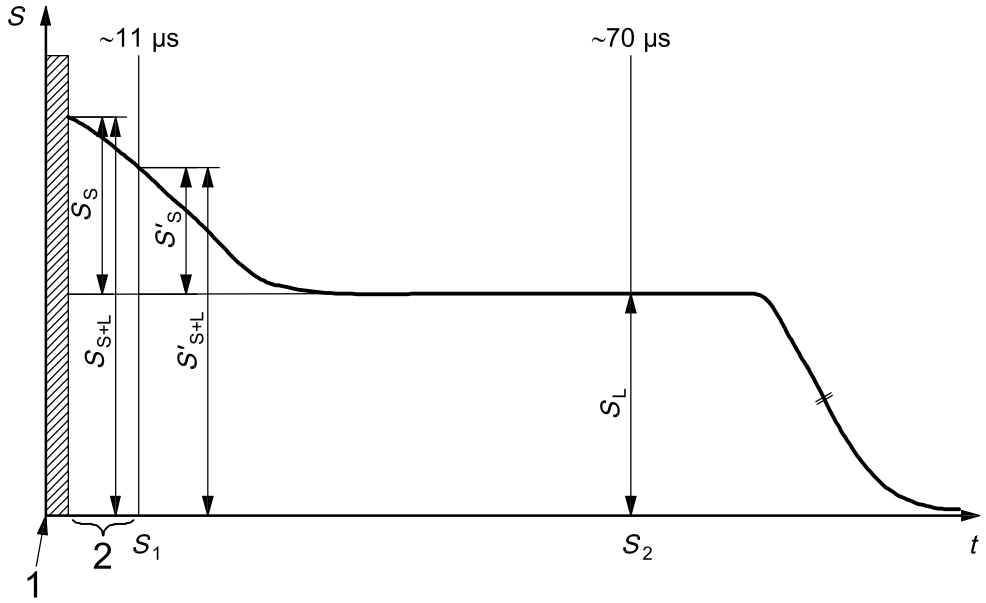
Table A.2 (*continued*)

	Palm oil/palm stearin blend (F)	Cocoa butter, soft Brazilian type (G)	Cocoa butter, standard West African type (H)	Illipe butter (Borneo tallow, Tengkawang fat) (I)	Cocoa butter equivalent, standard type, $w_{SFC,30} \approx$ 35 % to 40 % (J)	Palm mid-fraction, hard/CBE grade, (K)
Reproducibility standard deviation, s_R	0,90	0,25	0,38	0,36	0,24	0,06
Coefficient of variation of reproducibility, CV(R)	8,4	154,5	118,8	114,9	120,6	137,6
Reproducibility limit, R = 2,8 s_R	2,53	0,69	1,06	1,02	0,67	0,17
Determination temperature	45 °C					
No. participating laboratories	5					
No. laboratories retained after eliminating outliers	5					
No. test results, all labs	10					
Mean	5,20					
Repeatability standard deviation, s_r	0,35					
Coefficient of variation of repeatability, CV(r)	6,8					
Repeatability limit, r = 2,8s_r	0,99					
Reproducibility standard deviation, s_R	1,23					
Coefficient of variation of reproducibility, CV(R)	23,7					
Reproducibility limit, R = 2,8 s_R	3,45					

Annex B
(informative)

Theory of the direct method

A short radio-frequency pulse is applied which rotates the magnetic field through 90°, i.e. perpendicular to the prevailing magnetic field applied by the permanent magnet. Mainly due to spin-spin relaxation, the magnetization signal in the detector decays over several hundred milliseconds as shown in Figure B.1.



Key

1	pulse
2	dead time
S	magnetization decay signal
S_1	magnetization decay signal measured at about 11 µs
S_2	magnetization decay signal measured at about 70 µs
S_L	magnetization decay signal corresponding to liquid phase after about 70 µs
S_S	magnetization decay signal corresponding to solid phase at time 0
S'_S	magnetization decay signal corresponding to solid phase after about 11 µs
S_{S+L}	magnetization decay signal corresponding to both solid and liquid phases at time 0
S'_{S+L}	magnetization decay signal corresponding to both solid and liquid phases after about 11 µs
t	time

Figure B.1 — Decay of magnetization signal from a fat sample after application of a single 90° radio-frequency pulse

The decay of the signal from the protons in the solid state is rapid, occurring over tens of microseconds, whereas the decay of the signal from the protons in the liquid state is much slower, occurring over tens to hundreds of milliseconds. In practice, in a commercial bench-top instrument, the liquid signal will decay within a few milliseconds. By suitable electronics, it should then be possible to measure the solid plus liquid and the liquid signals separately and hence determine the SFC. However, as shown in Figure B.1, the instrument has a dead time after the pulse when no measurements can be made. Thus, the total signal S_{S+L} cannot be measured, but only S'_{S+L} after about 11 µs. The NMR spectrometer records two signals, S_1 and S_2, at about 11 µs and 70 µs, corresponding to S'_{S+L} and S_L, respectively.

For the direct method, a linear extrapolation from S'_{S+L} at about 11 µs to S_{S+L} at the unmeasurable time 0 is assumed so that:

$$S_S = f\,S'_S \tag{B.1}$$

where f is an extrapolation factor to be determined empirically.

Since

$$S_{S+L} = S_S + S_L \tag{B.2}$$

then

$$S_S = f(S'_{S+L} - S_L) \tag{B.3}$$

giving

$$w_{SFC,T} = \frac{f(S'_{S+L} - S_L)}{f(S'_{S+L} - S_L) + S_L} \times 100 \tag{B.4}$$

which is another expression of Equation (3).

The direct method gives only an approximate value of the SFC because:

a) a linear extrapolation is fundamentally wrong for a non-linear decay curve;

b) the value of f varies according to the molecular mobility, i.e. temperature, type of packing of the protons (in other words, the polymorphism), as well as the crystal size — for values given by a typical spectrometer, see Table B.1;

c) f varies with temperature as the liquid phase expands.

Table B.1 — Values of f according to polymorphism

Polymorphism	Value range for factor, f
α	1,10 to 1,30
β′	1,40 to 1,50
β	1,60 to 2,00

Nevertheless, the direct method is often the preferred method for routine use because of its good reproducibility and its relative simplicity of calibration. Since many fats for practical use exist mainly in the β′-polymorph, a value of f between 1,40 and 1,45 is normally used for all temperatures and is pre-set by calibration using plastic-in-oil standards.

Annex C
(informative)

Additional measurement protocols

Measurement protocol		Applicable to	Instrumental conditions	First time at 0 °C	Tempering		Second time at 0 °C	Measurement conditions	
No.	Name			min	Time min	Temp. °C		Type	Time min
3D	Slow crystallizing	Milk fat and its fractions and blends containing predominantly milk fat; tallows and their fractions, and blends containing predominantly tallow; other slow-crystallizing fats	f = 1,40 to 1,45; repetition time [a], t_{rep} = 2 s; No. pulses [b], n_p = 3	—	—		(16 ± 0,5) h	Parallel	(30 ± 2)
4D	AOCS solid fat index [c]	Fats and blends (comprising mainly vegetable fats, hydrogenated and/or inter-esterified) crystallizing in the β′–polymorph and as used for margarines, spreads, shortenings, and other general food applications	f = 1,40 to 1,45; repetition time [a], t_{rep} = 2 s; No. pulses [b], n_p = 3	(15 ± 1) [d]	(30 ± 1)	26,7	(15 ± 1) min	Series or parallel at 10,0 °C, 21,1 °C, 26,7 °C, 33,3 °C and 37,8 °C only	(45 ± 2)
5	Rapid	As for 1D, but where a faster method is required for production control purposes	f = 1,40 to 1,45; repetition time [a], t_{rep} = 2 s; No. pulses [b], n_p = 3	—	—		(30 ± 1) min	Parallel	(15 ± 1)
6	Ultra-rapid	As for 1D, but where a very fast method is required for production control purposes	f = 1,40 to 1,45; repetition time [a], t_{rep} = 2 s; No. pulses [b], n_p = 3	—	—		Substitute 1 min in liquid nitrogen [e]	Parallel	(30 ± 1)

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

[b] Data collected from each pulse are averaged by the instrument. Preferably, three pulses shall be 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] Follows the tempering applied in the AOCS dilatometric method to measure SFC as the so-called solid fat index (AOCS method Cd 10-57), and is used where the best correlation between SFC and SFI is required.

[d] Before the first 0 °C crystallization, hold at 26,7 °C for (15 ± 1) min.

[e] **CAUTION — Liquid nitrogen is a potentially hazardous material if used improperly, with the danger of severe frostbite. Follow the supplier's instructions for safe handling.**

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