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Oilseeds — Simultaneous determination of oil and moisture contents — Method using pulsed nuclear magnetic resonance spectrometry

Graines oléagineuses — Détermination simultanée de la teneur en huile et en eau — Méthode par spectrométrie par résonance magnétique nucléaire pulsée

Reference number
ISO 10565:1993(E)

Foreword

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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 10565 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 2, *Oleaginous seeds and fruits*.

Annex A of this International Standard is for information only.

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Oilseeds — Simultaneous determination of oil and moisture contents — Method using pulsed nuclear magnetic resonance spectrometry

1 Scope

This International Standard specifies a rapid method for the determination of the oil and moisture contents of commercial oilseeds, using a pulsed nuclear magnetic resonance (NMR) spectrometer.

The method has been tested on rapeseed.

It applies to rapeseed with a moisture content of less than 10 %. For seeds with higher moisture contents, drying is necessary before the oil content can be determined by pulsed NMR.

NOTE 1 This method is currently used for soya beans and sunflower seeds; this does not, however, preclude its applicability to other commercial seeds, the oil of which is liquid at the temperature of measurement.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 659:1988, *Oilseeds — Determination of hexane extract (or light petroleum extract), called "oil content"*.

ISO 664:1990, *Oilseeds — Reduction of laboratory sample to test sample.*

ISO 665:1977, *Oilseeds — Determination of moisture and volatile matter content.*

3 Principle

Insertion of the test sample into the magnetic field of a pulsed NMR spectrometer.

Application of an alternating electromagnetic field in the form of an intense 90° radiofrequency (RF) pulse which excites all the hydrogen nuclei. Recording of the free induction decay (FID) following the 90° pulse. The maximum amplitude of this signal is proportional to the total number of protons from the water and oil phases of the sample.

Application of a second RF pulse, a so-called 180° pulse, to produce a spin-echo signal when only the signal from the oil phase contributes to the FID.

NOTE 2 The maximum amplitude of this echo signal is proportional to the oil content. It varies with the sample temperature following a complex law. An increase in temperature decreases the measured value of the echo.

Calculation of the difference between the two amplitudes, which is proportional to the moisture content.

Automatic conversion of the measured signals, after suitable calibration of the apparatus, into percentages of oil or moisture.

NOTE 3 Simultaneous indications of the oil and moisture contents can be given by some spectrometers equipped with a minicomputer and a specific program.

4 Calibration samples

Calibration samples shall be homogeneous and free from impurities.

4.1 Samples for moisture-content calibration

In order to obtain a reliable calibration curve, it is recommended that the moisture contents of the calibration samples be between 6 % and 9 % for sunflower seeds and rapeseeds, and between 6 % and 12 % for soya beans.

The moisture content of seeds can vary depending on storage conditions. It is therefore necessary to determine it in accordance with ISO 665 just prior to calibration.

4.2 Samples for oil-content calibration

Samples of oilseeds shall be of the same species as the test samples and of similar fatty acid compositions (especially for the analysis of rapeseeds which are rich in erucic acid, or sunflower seeds which are rich in oleic acid). Oil content shall be determined using the reference method described in ISO 659.

5 Apparatus

5.1 Pulsed low-resolution NMR spectrometer, suitable for measurement of the oil content and moisture content of oilseeds, and having the following characteristics.

a) Proton resonance frequency: about 10 MHz.

b) Production of 90° and 180° RF pulses for the spin-echo method.

c) Magnetization time or pulse repetition time: 1 s.

d) Excitation time (e) or pulse time:

$e = 5$ μs approximately for the 90° pulse,

$e = 10$ μs approximately for the 180° pulse (i.e. twice as long as for the 90° pulse).

e) Dead time (t) = 20 μs.

f) Total dead time (T_t), comprising the excitation time (e) and the dead time (t), where:

$T_t = 25$ μs for 90° pulses,

$T_t = 30$ μs for 180° pulses,

$t = 20$ μs.

g) NMR signal sampling:

1) 50 μs after the 90° pulse on the FID,

2) 7 000 μs after the 90° pulse on the spin-echo.

h) Interval between the 90° and the 180° pulse: 3 500 μs.

Interval between the 180° pulse and the echo maximum: 3 500 μs (the echo maximum corresponds to the oil signal only).

i) Electronic bandwidth filter: low (10 kHz).

j) Number of NMR measurements per sample: 16 to 25 (i.e. the total time of measurement is between 16 s and 25 s).

k) Facility for storage of calibration curves (straight lines, least-squares method) of moisture and oil contents.

l) Homogeneity of magnetic field for a desirable sample volume of 35 ml.

m) Start system, which automatically starts the measurement after the introduction of the sample tube.

n) Temperature-regulated magnet, in order to maintain all the NMR parameters stable.

CAUTION — Remove metallic objects from the proximity of the NMR spectrometer.

5.2 Sample tubes, made of glass, suitable for use with the NMR spectrometer.

5.3 Analytical balance, electronic, with an accuracy of ± 0,01 g, by preference linked to the NMR spectrometer so that the sample mass is recorded directly by the NMR.

5.4 Drying oven, capable of being maintained at 103 °C ± 2 °C.

5.5 Dishes, made of glass or metal, of diameter 7 cm to 10 cm, and provided with lids.

5.6 Desiccator, containing an efficient desiccant.

6 Sampling

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

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 542.[1]

7 Preparation of test sample

Prepare the test sample in accordance with ISO 664.

Remove from the prepared test sample all metallic objects (e.g. staples, needles, etc.). Whole seeds shall

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[1] ISO 542:1990, *Oilseeds — Sampling.*

be homogeneous and, as far as possible, free from impurities.

8 Calibration procedure

8.1 General

8.1.1 Use the set-up parameters of the NMR spectrometer (5.1) recommended by the manufacturer and optimize them by preliminary tests. For all calibration and measurement operations, follow the user's manual. Ensure that all operations during calibration and measurement are carried out under the same conditions and, in particular, at the same temperature (± 2 °C).

A minimum of three calibration samples is necessary, although more than three samples may be used.

8.1.2 Enter the parameters for the measurements (pulse sequence, attenuation, etc.) of the oil or moisture content (as applicable) into the NMR spectrometer following the manufacturer's recommendations, and specify a number under which the calibration curve is to be stored. The total measurement time shall be a minimum of 16 s.

8.1.3 Set the apparatus to the calibration mode.

8.1.4 Introduce a portion of the first calibration sample into a tared sample tube (5.2) up to the optimum height specified by the manufacturer and transfer the sample mass from the balance to the NMR apparatus.

NOTE 4 Manual feed of the mass of the calibration sample into the NMR spectrometer is also possible.

8.1.5 Enter, as applicable, the value of either the moisture content (as a percentage by mass) or the oil content (as a percentage by mass) into the spectrometer.

8.1.6 Introduce the sample tube containing the first calibration sample into the measuring head and determine the moisture or oil content.

8.1.7 Repeat the steps 8.1.4 to 8.1.6 for the two (or more) other calibration samples.

8.1.8 Calculate automatically or manually the calibration parameters of the calibration curve.

The correlation coefficient shall normally be greater than 0,95. If this is not the case, check the values obtained using the reference methods specified in ISO 659 and ISO 665 respectively, or repeat the calibration procedure using three (or more) other calibration samples.

NOTE 5 The calibration curve is stored under the storage number chosen in step 8.1.2.

8.1.9 Complete the oil-content calibration curve by using an empty tube and by repeating steps 8.1.4 to 8.1.6. This part of the method is not applicable to the moisture-content calibration curve.

8.2 Calibration procedure for the determination of moisture content

8.2.1 Procedure A: Three calibration samples with known moisture contents

To obtain a reliable calibration graph, it is necessary that the moisture contents of the calibration samples span the largest possible range. However, a moisture content of 0 is not permissible.

Follow the general procedure specified in 8.1.1 to 8.1.8.

8.2.2 Procedure B: One calibration sample with known moisture content

NOTE 6 In this calibration procedure, the quantity of moisture detected by the NMR spectrometer is varied by varying the height to which the sample tubes are filled with the calibration sample.

8.2.2.1 Follow the general procedure specified in 8.1.1 to 8.1.3.

8.2.2.2 Introduce into three tared sample tubes (5.2) portions of the calibration sample to approximate heights of 35 mm, 30 mm and 25 mm (20 mm minimum) and weigh them. Note the corresponding masses in grams as m_1, m_2 and m_3.

NOTE 7 Three calibration points is the minimum for establishing the calibration curve.

8.2.2.3 Calculate the relative moisture content, w, as a percentage by mass, of each of the three portions of the calibration sample as follows:

$$w_1 = \frac{m_1 \times W}{m_1}$$

$$w_2 = \frac{m_2 \times W}{m_1}$$

$$w_3 = \frac{m_3 \times W}{m_1}$$

where

m_1, m_2 and m_3	are the masses, in grams, of the three portions of the calibration sample in each of the three tubes, where m_1 is the largest mass;
W	is the moisture content, as a percentage by mass, of the cali-

bration sample, determined in accordance with ISO 665.

8.2.2.4 Enter into the NMR spectrometer the value, m_1, of the mass of the first portion.

8.2.2.5 Enter into the NMR spectrometer the value, w_1, of the relative moisture content of the first portion.

8.2.2.6 Insert the tube containing the first portion of the calibration sample into the measuring head and carry out the determination.

8.2.2.7 Repeat the operations described in 8.2.2.4 to 8.2.2.6 with the two other portions of the calibration sample, always entering m_1 in step 8.2.2.4, then the respective relative moisture contents w_2 and w_3 in step 8.2.2.5.

8.2.2.8 Calculate the calibration parameters in accordance with step 8.1.8.

8.3 Calibration procedure for the determination of oil content

8.3.1 Procedure A: Three calibration samples with known oil contents

Use the samples specified in 4.1.

Follow the general procedure specified in 8.1.1 to 8.1.9.

8.3.2 Procedure B: One calibration sample with known oil content

NOTE 8 In this calibration procedure, the quantity of oil detected by the NMR spectrometer is varied by varying the height to which the sample tubes are filled with the calibration sample.

8.3.2.1 Follow the general procedure specified in 8.1.1.

8.3.2.2 Introduce into three tared sample tubes (5.2) portions of the calibration sample with known oil content to approximate heights of 35 mm, 30 mm and 25 mm (20 mm minimum) and weigh. Note the corresponding masses in grams as m_1, m_2 and m_3.

8.3.2.3 Calculate the relative oil content, h, as a percentage by mass, in the three portions of the calibration sample as follows:

$$h_1 = \frac{m_1 \times H}{m_1}$$

$$h_2 = \frac{m_2 \times H}{m_1}$$

$$h_3 = \frac{m_3 \times H}{m_1}$$

where

m_1, m_2 and m_3 are the masses, in grams, of the three portions of the calibration sample;

H is the oil content, as a percentage by mass, of the calibration sample, determined in accordance with ISO 659.

8.3.2.4 Follow the general procedure specified in 8.1.2 to 8.1.8, entering the oil content, in grams, in step 8.1.5.

8.3.2.5 Enter into the NMR spectrometer the value, m_1, of the mass of the first portion.

8.3.2.6 Enter into the NMR spectrometer the value, h_1, of the relative oil content of the first portion.

8.3.2.7 Insert the tube containing the first portion of the calibration sample into the measuring head and carry out the determination.

8.3.2.8 Repeat the operations described in 8.3.2.5 to 8.3.2.7 with the two other portions of the calibration sample, always entering m_1 in step 8.3.2.5, then the respective relative oil contents h_2 and h_3 in step 8.3.2.6.

8.3.2.9 To obtain the zero-point on the calibration curve, enter into the NMR spectrometer the value m_1 of the mass and a relative oil content of 0,01 %. Insert an empty tube into the measuring head and carry out the determination.

8.3.2.10 Calculate the calibration parameters in accordance with 8.1.8.

9 Procedure

Carry out at least two determinations on test portions taken from the same test sample.

9.1 Requirements for the test room and conditioning of the test sample

The temperature of the test room shall be maintained between 17 °C and 28 °C. Therefore, it is recommended that the test room be temperature controlled.

Transfer the test samples (clause 7) to the test room at least 60 min before the determination to allow them to reach equilibrium temperature.

9.2 Test portion

NOTE 9 The reproducibility of the method is improved if the NMR measurements are carried out on test portions of constant volume.

Prepare test portions of constant volume using one of the following methods.

a) Measure a chosen volume of the test sample (9.1) and then transfer quantitatively this volume to a tared sample tube (5.2) (e.g. use 40 ml of test sample for sample tubes 40 mm in diameter).

b) Transfer a sufficient quantity of the conditioned test sample (9.1) to the sample tube to fill it to an optimum height of 30 mm ± 5 mm. (It is recommended that the sample tubes be marked at a height of 30 mm using a felt-tipped pen.)

If carrying out measurements on dehulled sunflower seeds, ensure that they are well distributed in the sample tubes.

NOTES

10 For seed samples where only small quantities are available, it is possible to use smaller sample tubes with a diameter of 25 mm. A measuring volume of 13 ml, corresponding to about 9 g of rapeseed is required for them. Owing to the short time of measurement and the small sample quantity a three-fold test is recommended in order to obtain a representative result, and in this case the variability is greater.

11 For a filling height of 30 mm, the following ranges of masses are recommended:

whole rapeseeds: 22 g to 25 g

whole sunflower seeds: 14 g to 17 g

whole soya beans: 21 g to 24 g.

9.3 Determination

IMPORTANT — Check the calibration prior to each series of measurements (or at least once a day), using a calibration sample of known oil and moisture contents.

9.3.1 General case

9.3.1.1 Select the program and the instrument parameters for the moisture or oil content, as applicable, and the number of the calibration curve corresponding to the intended test and to the species of seeds.

9.3.1.2 Set the apparatus to the measurement mode.

9.3.1.3 Weigh the test portion (9.2) and transfer the value of the mass from the balance to the NMR spectrometer.

9.3.1.4 Insert the sample tube containing the test portion into the measuring head and determine the moisture and oil contents.

9.3.2 Rapeseeds and sunflower seeds having a moisture content greater than 10 % (*m/m*) and soya beans having a moisture content greater than 14 % (*m/m*)

NOTE 12 In this case, the pulsed NMR and the spin-echo method do not give a correct value because the excess moisture has a different behaviour and contributes together with the oil to the spin-echo signal. This excess moisture has therefore to be removed by drying.

9.3.2.1 Transfer quantitatively the previously weighed test portion of mass m into dishes (5.5), with the lids removed, and dry in the oven (5.4) set at 103 °C for 1 h.

9.3.2.2 Remove the dishes from the oven, cover them with a lid and allow them to cool in the desiccator (5.6). Check that they have reached the measuring temperature (approximately 1 h).

9.3.2.3 Transfer quantitatively the dried test portion to a previously weighed sample tube, weigh and deduce the mass, m_0, of the dried test portion.

9.3.2.4 Follow the procedure specified in 9.3.1.1 and 9.3.1.2.

9.3.2.5 Enter the value of the original mass, m, of the test portion into the NMR spectrometer.

9.3.2.6 Insert the sample tube containing the test portion into the measuring head and determine the moisture content, W_b.

9.3.2.7 Correct the reading obtained for the moisture content, W_b, to give the total moisture content W, as a percentage by mass, using the following formula:

$$W = W_b + \frac{m - m_0}{m} \times 100$$

where

m is the original mass, in grams, of the test portion, determined in 9.3.2.1;

m_0 is the mass, in grams, of the dried test portion, determined in 9.3.2.3;

W_b is the moisture content, expressed as a percentage by mass, measured in 9.3.2.6.

10 Expression of results

Check whether the repeatability requirement (11.1) is satisfied.

If so, take as the result the arithmetic mean of the results of the two or more determinations. If not, reject the results and carry out two further determinations.

11 Precision

For the results of an interlaboratory test carried out on rapeseeds, see annex A.

11.1 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, should not be greater than

for oil: 0,6 %

for moisture: 0,1 %

11.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than

for oil: 1,6 %

for moisture: 0,7 %

12 Test report

The test report shall specify

- the NMR spectrometer used,
- the method used,
- the test result(s) obtained, and
- if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may hav influenced the test result(s).

The test report shall include all information necessary for the complete identification of the sample.

Annex A

(informative)

Results of interlaboratory test

An interlaboratory test at the international level was carried out by 13 laboratories. The statistical results indicated in table A.1 were determined in accordance with ISO 5725.[2]

Interlaboratory tests are presently under way for soya beans, sunflower seeds and linseeds.

Table A.1 — Interlaboratory test on rapeseed

	Oil content		**Moisture content**	
	Batch 1	**Batch 2**	**Batch 1**	**Batch 2**
Number of laboratories retained after eliminating outliers	13	13	12	12
Mean value of content, % (*m/m*)	40,83	41,38	7,01	6,01
Standard deviation of repeatability, s_r	0,25	0,15	0,04	0,03
Coefficient of variation of repeatability, %	0,63	0,38	0,60	0,58
Repeatability, 2,83 s_r	0,72	0,45	0,11	0,09
Standard deviation of reproducibility s_R	0,55	0,55	0,24	0,36
Coefficient of variation of reproducibility, %	1,35	1,33	3,41	6,01
Reproducibility, 2,83 s_R	1,56	1,56	0,67	1,02

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[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*