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Oilseeds — Determination of oil content (Reference method)

Graines oléagineuses — Détermination de la teneur en huile (Méthode de référence)

Reference number
ISO 659:2009(E)

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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 659 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 2, *Oleaginous seeds and fruits and oilseed meals*.

This fourth edition cancels and replaces the third edition1) (ISO 659:1998), which has been technically revised. The main change is the inclusion of an additional subclause (Subclause 8.3.5) for the preparation of the test sample in the case of sunflower seed. This different procedure for sunflower seed includes an extra step, viz measurement of the moisture content after grinding the seed. This is necessary to correct for the loss of moisture caused by the heating of the seed which occurs during grinding due to the particular physical nature of sunflower seed.

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1) Users should note that the front cover and foreword of ISO 659:1998 indicate erroneously that it is the second edition, whereas it is in fact the third.

INTERNATIONAL STANDARD **ISO 659:2009(E)**

Oilseeds — Determination of oil content (Reference method)

1 Scope

This International Standard specifies a reference method for the determination of the hexane extract (or light petroleum extract), called the “oil content”, of oilseeds used as industrial raw materials. The procedure for sunflower seed is different from those for other seeds as it includes an additional moisture content determination after the seed has been ground to prepare the test sample.

The method has been tested on rapeseed, soya beans and sunflower seed. This does not, however, preclude its applicability to other commercial seeds.

If required, the pure seeds and the impurities (see 9.4) can be analysed separately. In the case of groundnuts (see 10.1.6), the pure seeds, the total fines, the non-oleaginous impurities and the oleaginous impurities can be analysed separately.

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 658, *Oilseeds — Determination of content of impurities*

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

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

3 Terms and definitions

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

3.1
hexane extract
“oil content”
all the substances extracted under the operating conditions specified in this International Standard, expressed as a percentage by mass of the product as received, or on the cleaned seed

NOTE On request, it may be expressed relative to the dry matter.

4 Principle

The oil is extracted from a test portion, in a suitable apparatus, with hexane or light petroleum. The solvent is removed from the extract and the extract weighed. Due to the varied nature of seeds, there are some variations in the procedure for different seeds.

5 Reagent

5.1 Technical hexane, *n*-hexane or **light petroleum**, essentially composed of hydrocarbons with six carbon atoms, of which less than 5 % distils below 40 °C and more than 95 % distils between 40 °C and 60 °C or between 50 °C and 70 °C, and which has a bromine value of less than 1. The residue on complete evaporation shall not exceed 2 mg per 100 ml.

6 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.1 Analytical balance, with an accuracy of ± 0,001 g.

6.2 Mechanical mill, easy to clean, appropriate for the nature of the oilseeds and allowing the oilseeds to be ground to a uniform particle size without appreciable heating or change in moisture, volatile-matter or oil content.

NOTE The following mills have been found to give satisfactory results:

- the Christy & Norris 8" laboratory mill 2), with perforated-plate or bar-type screens depending on seed type (e.g. 0,8 mm perforated plate, 3 mm and 6 mm bar screens; see 8.3.2, 8.3.3 and 8.3.4);
- the Retsch ZM 200 ultra-centrifugal mill 2), with a 1 mm screen for sunflower seed or with other screens depending on seed type.

Other mills may be satisfactory, provided they conform to the criteria given above.

6.3 Mechanical microgrinder, capable of producing a fineness of grind of the oilseeds of less than 160 µm, with the exception of the “shell”, particles of which may reach 400 µm. Coffee grinders and similar cutting-action mechanical grinders shall not be used as microgrinders.

NOTE A ball mill comprising a stainless-steel tube of volume approximately 120 ml and 1 cm, 2 cm and 3 cm diameter steel balls (weighing approximately 7 g, 30 g and 130 g), depending on the sample being ground, and utilizing extremely rapid shaking, has been found to be suitable.

If the microgrinder and its closure are designed for use with solvents, a small amount (approximately 20 ml) of solvent may be added to the cylinder in order to increase the speed of the extraction.

6.4 Extraction thimble (cellulose) and **cotton wool**, free from matter soluble in hexane or light petroleum.

6.5 Suitable extraction apparatus, fitted with a flask of capacity 200 ml to 250 ml.

Straight-through extractors, for example the Butt, Smalley or Bolton-Williams type, are preferred for safety reasons and have been found to give a more efficient extraction.

The use of other extractors is conditional upon the results of a test on a standard material of known oil content to confirm the suitability of the apparatus. If a Soxhlet syphoning-type extractor is used then, for safety reasons, a 250 ml flask should be utilized and the volume of solvent used shall be at least 150 ml. This should prevent rapid boiling on syphoning, which could otherwise cause loss of solvent and/or part of the test portion.

6.6 Pumice stone, in small particles, or other anti-bumping granules, previously dried in an oven at (130 ± 2) °C and cooled in a desiccator.

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2) The Christy & Norris 8" mill and the Retsch ZM 200 mill are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

6.7 Apparatus for safely removing solvent from extraction thimble (for example, in a current of inert gas).

WARNING — This process should be carried out in a fume hood.

6.8 Electric heating bath (sand bath, water bath, heating mantle, etc.) or **hot-plate**.

6.9 Electrically heated oven, with thermostatic control, capable of being maintained at (103 ± 2) °C. The oven shall be capable of being used at either atmospheric or reduced pressure (see 9.3.3).

6.10 Desiccator, containing an efficient desiccant (e.g. silica gel, with orange indicator, or P_2O_5).

6.11 Electrically heated oven, capable of being maintained at (130 ± 2) °C (for cottonseed, see 8.3.6).

6.12 Metal dish, flat-bottomed, of diameter 100 mm and height approximately 40 mm.

7 Sampling

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

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

8 Preparation of test sample

8.1 Reduction of laboratory sample

Prepare the test sample in accordance with ISO 664. If large non-oleaginous foreign bodies have been separated before reduction of the laboratory sample, make allowance for this in the calculation (see 10.1.5). Depending on the requirements of the contract or the instructions, take the sample as received or after separation of the impurities.

8.2 Predrying

8.2.1 The moisture content of the test portion (see 9.2) shall be less than a mass fraction of 10 % when extraction of the oil (see 9.3) is begun.

NOTE Failure to observe this requirement can result in an incorrect result and invalidate the analysis.

Use a rapid screening method to assess the moisture content of the laboratory sample (see 8.1). If the moisture mass fraction is greater than 10 %, reduce it to less than 10 % by drying the test sample (see 8.1) in a dish in an oven maintained at a temperature not greater than 80 °C. Store the partially dried material in an air-tight jar. Determine the moisture contents of the partially dried test sample and the original sample in accordance with the method given in ISO 665 and make allowance for this in the final calculation (see 10.1.8).

8.3 Test sample

8.3.1 Preliminary remarks

It is essential that oil extractions are carried out within 30 min of grinding, especially if the free fatty acid content of the extracted oil is to be determined.

Care shall be taken to clean all mills thoroughly before and after each sample has been ground. Any material adhering to the mill shall be incorporated into the bulk of the ground material.

NOTE In the following text, “seed” or “kernels” implies both whole seeds or kernels and broken fragments of them.

8.3.2 Palm kernels

Shell is considered to be part of the impurities, even when attached to the kernels. Shell and dirt shall be separated quantitatively from the kernels before analysis.

Shell is hard and thus difficult to mill. It is almost impossible to obtain a homogeneous sample if kernels and impurities are milled together. If the oil content of the whole sample, as received, is required, analyse the kernels and impurities (shell and dirt) separately and calculate the oil content (see 10.1.3).

Prepare the test sample by grinding, separately, 600 g of the well-mixed kernels and all of the shell and dirt separated during the quantitative determination of impurities in accordance with the method given in ISO 658.

The following milling techniques have been found to be suitable:

- for pure kernels: a mechanical mill (6.2) with a 6 mm bar screen;
- for shell and dirt: grind for 10 min in a microgrinder (6.3) using a 3 cm steel ball.

8.3.3 Copra (and coconut)

Freeze the entire sample before milling in the mechanical mill (6.2).

A mechanical mill with a 6 mm screen has been found to be satisfactory. The length of the particles shall be approximately 2 mm but shall not be greater than 5 mm. Mix the particles carefully and carry out the determination without delay.

Care shall be taken to avoid condensation of moisture onto the seeds during and after grinding.

8.3.4 Large- and medium-sized seeds (illipe nuts, shea nuts, groundnuts, soya, etc., but NOT sunflower seed or cottonseed)

Grind the test sample in the mechanical mill (6.2) until the major dimension of the particles obtained is not greater than 2 mm. Reject the first particles (about one-twentieth of the sample), collect the rest, mix carefully and carry out the determination without delay.

The following milling techniques have been found to be satisfactory:

- for soya: a mechanical mill with a 0,8 mm perforated plate or with a 1 mm screen;
- for groundnuts: a mechanical mill with a 3 mm bar screen (with samples containing a mass fraction of more than about 45 % of oil, care shall be taken to avoid, as far as possible, the formation of a pasty mass);
- other seeds: a mechanical mill with a 6 mm bar screen.

The grinding of very oily seeds can often be improved if the seeds are first frozen at –10 °C to –20 °C, but great care shall be taken to avoid condensation of moisture onto the seeds during and after grinding.

8.3.5 Sunflower seed

Determine the moisture and volatile-matter content of the sunflower seed (as received) on the whole seed in accordance with ISO 665, taking care to observe strictly the drying periods laid down in the method specified in ISO 665. Record the result of this moisture and volatile-matter determination (U_1).

Grind the test sample in a mechanical mill (6.2). During grinding, the mill shall be fed slowly with the sunflower seeds to prevent a paste being formed. A mechanical mill with a 1 mm screen has been found to be suitable. If a screen-type mill is not used, sieve through a 2 mm sieve and regrind the material which does not pass through the sieve until the major dimension of the particles is not greater than 2 mm. Collect all the sievings, mix carefully and, without delay, determine the moisture and volatile-matter content in accordance with ISO 665, taking care to observe strictly the drying periods laid down in ISO 665.

Record the result of the moisture and volatile-matter determination on the ground seed (U_2) and carry out the oil extraction on the ground seed as specified in Clause 9.

8.3.6 Cottonseed

Weigh, to the nearest 1 mg, into a tared metal dish (6.12) about 15 g of the sample as received. Place the dish and seeds in the oven (6.11), previously heated to 130 °C, and leave to dry for 2 h at (130 ± 2) °C. Then remove the dish from the oven and allow to cool in air for about 30 min. Transfer the whole test portion to a mill (6.2) and grind to break up the seeds and linters. Transfer the ground material to an extraction thimble (6.4) and proceed with the determination.

8.3.7 Small seeds (linseed, rapeseed, etc.)

Take a representative portion of approximately 100 g from the sample as received and grind it in a mechanical mill (6.2) so that no whole seeds remain. Ensure that any fine material remaining in the mill is incorporated into the bulk of the ground material and that the whole is thoroughly mixed. Care shall be taken that moisture is not lost from the ground test sample.

The grinding time and speed (if variable) shall be predetermined for a particular mill and seed. The grinding shall not cause segregation of the “meat” and “husks” of the seed, the test sample shall not be oily and at least a mass fraction of 95 % shall pass a 1 mm sieve.

If the oil content in the seed free from impurities is required, remove the impurities by the method given in ISO 658 and prepare at least 30 g of “clean” seed (including broken seed), as it is impracticable to prepare 100 g of very small seed such as sesame seed.

9 Procedure

9.1 General

Carry out two single determinations in accordance with 9.2 and 9.3 and check that the requirement concerning repeatability (see 11.2) is satisfied. If it is not, repeat the determination on two further test portions taken from a freshly prepared test sample (see 8.3). If the differences still exceed the value stated in 11.2, take as the result the arithmetic mean of the four determinations carried out, provided that the maximum difference between the individual results does not exceed 1,50 % in absolute value.

9.2 Test portion

9.2.1 Weigh, to the nearest 1 mg, (10 ± 0,5) g of the ground test sample (see 8.3). For treatment of impurities, see 9.4.

9.2.2 Transfer the test portion within 30 min of grinding to the thimble (6.4), using a small wad of cotton wool (see 6.4) moistened with solvent (5.1) to transfer the last traces of ground seed from the weighing container to the thimble. Use this cotton wool to plug the thimble.

9.3 Determination

9.3.1 Preparation of flask

Weigh, to the nearest 1 mg, a flask (6.5), containing a few granules of pumice stone (6.6), which has been previously dried in an oven and cooled in a dessicator.

9.3.2 Solvent extraction

9.3.2.1 General

The times stipulated for the three extractions (see 9.3.2.2, 9.3.2.3 and 9.3.2.4) may be varied slightly (for example by ± 10 min). On no account shall extended extraction times (for example overnight) be employed.

9.3.2.2 First extraction

Place the thimble (6.4) containing the test portion in the extraction apparatus (6.5). Pour into the flask the necessary quantity of solvent (5.1). Fit the flask to the extraction apparatus on the electric heating bath or hot-plate (6.8). Carry out the heating so that the rate of reflux is at least 3 drops per second (boiling moderately, not violently).

After extracting for 4 h, allow to cool. Remove the thimble from the extraction apparatus and place it in a current of air in order to expel the greater part of the residual solvent (see 6.7).

9.3.2.3 Second extraction

Empty the contents of the thimble into the microgrinder cylinder (see 6.3) and grind for 7 min. For most seeds, six 1 cm diameter steel balls in a 150 ml cylinder have been found to be satisfactory; for cottonseed with adherent linters, three 2 cm balls are satisfactory.

Put the mixture back into the thimble, using a small piece of cotton wool to remove any residual seed particles from the grinding apparatus. Add this to the thimble. Put the thimble back into the extraction apparatus and re-extract for a further 2 h, using the same flask containing the first extract. Allow to drain and cool, remove the thimble again and eliminate most of the solvent in the same way as before (see 9.3.2.2).

9.3.2.4 Third extraction

Repeat the grinding as above. Put the mixture back into the thimble, cleaning the grinding apparatus as before (see 9.3.2.3), and put the thimble back into the extraction apparatus. Extract as before (see 9.3.2.2) for 2 h, using the same flask.

9.3.3 Removal of solvent and weighing of the extract

Remove the greater part of the solvent from the flask by distillation on the electric heating bath or hot-plate. Assist the removal of solvent by blowing air or, preferably, an inert gas (such as nitrogen or carbon dioxide) into the flask for short periods. Remove the last traces of solvent by heating the flask for between 30 min and 60 min in the oven (6.9) at (103 ± 2) °C at atmospheric pressure or at 80 °C under vacuum.

In the case of oil seed rich in volatile acids (copra, palm kernels, etc.), drying of the extract shall be carried out at atmospheric pressure and at 80 °C maximum.

In the case of drying or semi-drying oils, it is preferable to remove the residual solvent by drying under reduced pressure.

In the case of non-lauric oils, when a vacuum oven is available, traces of solvent may be removed by heating at 80 °C under vacuum.

Allow the flask to cool in the desiccator (6.10) to ambient temperature, but for at least 1 h, and weigh to the nearest 1 mg.

Reheat in the oven (6.9) at (103 ± 2) °C at atmospheric pressure or at 80 °C under vacuum for between 20 min and 30 min, allow to cool and reweigh.

The difference between the two weighings shall not exceed 5 mg. If it does, repeat the operations of heating, cooling and weighing until the difference between two successive weighings does not exceed 5 mg. Note the final mass of the flask.

If there is a significant increase in mass (over 5 mg), oxidation of a drying oil may be taking place and a further determination shall be carried out, taking precautions to exclude oxygen.

9.3.4 Impurities content of the extracted oil

The oil extracted shall be clear; if it is not, determine the impurities content. For this purpose, dissolve the fatty matter in the solvent used for extraction. Then filter through a filter paper, previously dried at (103 ± 2) °C to constant mass. Wash the filter paper several times with the same solvent to remove the oil completely. Dry again at (103 ± 2) °C to constant mass. Cool and weigh the filter paper in a suitable vessel provided with a lid. Correct the result accordingly.

9.4 “Oil content” of impurities

To determine the “oil content” of the impurities, carry out the analysis in the same manner as for the seeds but with the following differences:

- the test portion may be 5 g to 10 g;
- only one extraction, for a period of 4 h, is necessary, the small error thus introduced into the “oil content” of the product as received being negligible.

10 Expression of results

10.1 Method of calculation

10.1.1 General

In all cases, take as the final result the arithmetic mean of the two determinations (see 9.1), expressed to one decimal place.

10.1.2 Determination on product as received (except sunflower seed)

The “oil content” w, expressed as a percentage by mass of the product as received, is given by:

$$w = \frac{m_2}{m_1} \times 100$$

where

m_1 is the mass, in grams, of the test portion (see 9.2);

m_2 is the mass, in grams of the dried extract (see 9.3.3).

10.1.3 Determination of "oil content" on product as received for sunflower seed

The "oil content" w of sunflower seed, expressed as a percentage by mass of the product as received, is given by:

$$w = \frac{m_2 \times (100 - U_1)}{m_1 \times (100 - U_2)} \times 100$$

where

m_1 is the mass, in grams, of the test portion (see 9.2);

m_2 is the mass, in grams, of the dried extract (see 9.3.3);

U_1 is the percentage by mass of water and volatile matter determined on the whole seed as received (see 8.3.5) in accordance with ISO 665;

U_2 is the percentage by mass of water and volatile matter determined on the ground test portion (see 8.3.5) in accordance with ISO 665.

10.1.4 Palm kernels

The oil (and free fatty acid) contents of the product as received are calculated from separate analyses of the seeds and the impurities.

The formula given in 10.1.2 is used to calculate the "oil content" of both the seeds and the impurities when the seeds and the impurities are analysed separately (see 9.4).

In this case, the "oil content" w_0, expressed as percentage by mass of the product as received (seeds and impurities), is given by:

$$w_0 = w_1 - \left[\frac{P}{100} \left(w_1 - w_2 \right) \right]$$

where

w_1 is the percentage, by mass, of oil in the pure seeds;

w_2 is the percentage, by mass, of oil in the impurities;

P is the percentage, by mass, of impurities in the product as received.

10.1.5 Case where large non-oleaginous foreign bodies have been separated before the analysis (see 8.1)

In this case, the "oil content" w_n, expressed as percentage by mass of the product as received, is given by:

$$w_\text{n} = w \times \left[\frac{100 - x}{100} \right]$$

where

w is the percentage, by mass, of oil in the product as received (calculated in accordance with 10.1.2 or 10.1.3, as appropriate);

x is the percentage, by mass, of large non-oleaginous foreign bodies previously separated from the original product as received.

10.1.6 Groundnuts

The “oil content” w, expressed as percentage by mass of the product as received, is given by:

$$w = w_1 - \left[\frac{p + I_o + I_n}{100} \right] (w_1 - w_2)$$

where

p is the percentage, by mass, of total fines;

I_o is the percentage, by mass, of oleaginous impurities;

I_n is the percentage, by mass, of non-oleaginous impurities;

w_1 is the percentage, by mass, of oil in the pure seeds;

w_2 is the percentage, by mass, of oil in the impurities.

If the test has been carried out on the sample as received, calculate the “oil content” in accordance with 10.1.2.

10.1.7 “Oil content” expressed in relation to the dry matter

The “oil content” w_d, expressed as a percentage by mass of the dry matter, is given by:

$$w_d = w_0 \times \left[\frac{100}{100 - U} \right]$$

where

w_0 is the percentage, by mass, of oil in the product as received;

U is the percentage, by mass, of water and volatile matter, determined in accordance with ISO 665.

10.1.8 “Oil content” expressed on the basis of any specified moisture content

It may by necessary to convert the “oil content” of a sample, determined at one moisture content, to that at another moisture content, for example if a sample has been partially dried before the test sample has been weighed out. In this case:

$$w' = w \times \left[\frac{100 - U'}{100 - U} \right]$$

where

w is the “oil content” at moisture and volatile-matter content U;

w' is the “oil content” at moisture and volatile-matter content U'.

11 Precision

11.1 Interlaboratory test programme

Details of an interlaboratory test programme on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test programme may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained with 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 not be greater than the values of r given in Table A.1 in more than 5 % of cases.

11.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, will not be greater than the values of R given in Table A.1 in more than 5 % of cases.

12 Test report

The test report shall contain at least the following information:

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

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) the solvent used;

e) all operating conditions not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the result;

f) the test result obtained, indicating clearly whether the result represents the “oil content” of the product as received or the “oil content” in relation to the dry matter of the seeds or kernels from which the impurities have been removed or to a specific moisture or dry-matter content;

g) if the repeatability was checked, the final result obtained.

Annex A
(informative)

Results of interlaboratory tests on the determination of oil content

Interlaboratory tests were carried out at the international level by the Federation of Oils, Seeds and Fats Associations Limited (FOSFA) in accordance with ISO 5725-1 and ISO 5725-2 on rapeseed (2000), soya beans (1998) and sunflower seed (2001 and 2005). The results are shown in Table A.1.

Table A.1 — Results of interlaboratory tests

Parameter	Rapeseed	Soya beans	Sunflower seed	Sunflower seed
Number of participating laboratories	29	42	51	59
Number of laboratories retained after eliminating outliers	29	40	51	54
Number of test results in all laboratories	58	80	102	108
Mean “oil content” value, % by mass	43,06	19,38	44,10	45,52
Repeatability standard deviation, s_r	0,10	0,07	0,13	0,14
Repeatability coefficient of variation, CV(r)	0,23	0,38	0,29	0,31
Repeatability limit, r	0,27	0,20	0,36	0,40
Reproducibility standard deviation, s_R	0,55	0,38	0,49	0,62
Reproducibility coefficient of variation, CV(R)	1,28	1,97	1,12	1,36
Reproducibility limit, R	1,54	1,07	1,38	1,73

Bibliography

[1] ISO 542, *Oilseeds — Sampling*

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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