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**Animal and vegetable fats and oils —  
Determination of refractive index**

*Corps gras d'origines animale et végétale — Détermination de l'indice de réfraction*

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6320 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This fourth edition cancels and replaces the third edition (ISO 6320:1995), which has been revised by the addition of precision data.

Annex A of this International Standard is for information only.

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# Animal and vegetable fats and oils — Determination of refractive index

## 1 Scope

This International Standard specifies a method for the determination of the refractive index of animal and vegetable fats and oils.

## 2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

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## 3 Terms and definitions

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For the purposes of this International Standard, the following term and definition apply.

### 3.1

#### refractive index (of a medium)

ratio of the velocity of light of a definite wavelength in a vacuum to its velocity in the medium

NOTE 1 In practice, the velocity of light in air is used in place of that in a vacuum and, unless otherwise specified, the selected wavelength is the mean wavelength of the sodium D lines (589,6 nm).

NOTE 2 The refractive index of a given substance varies with the wavelength of the incident light and with temperature. The notation used  $n_D^t$ , where  $t$  is the temperature in degrees Celsius.

## 4 Principle

By means of a suitable refractometer, the refractive index of a liquid sample is measured at a specified temperature.

## 5 Reagents

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

**5.1 Ethyl laurate**, of quality suitable for refractometry, and of known refractive index.

**5.2 Hexane**, or other suitable solvents, such as **light petroleum**, **acetone** or **toluene**, for cleaning the prism of the refractometer.

## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

**6.1 Refractometer**, for example of the Abbé type, suitable for measurements of refractive index to within  $\pm 0,000 1$  over the range  $n_D = 1,300$  to  $n_D = 1,700$ .

**6.2 Light source: sodium vapour lamp**

White light can also be used if the refractometer is fitted with an achromatic compensation system.

**6.3 Glass plate**, of known refractive index.

**6.4 Water bath**, thermostatically controlled, with a circulation pump, and capable of being maintained to the nearest  $\pm 0,1$  °C.

**6.5 Water bath**, capable of being maintained at the temperature at which the measurements are to be made (in the case of solid samples).

## 7 Sampling

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

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

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## 8 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

The refractive index shall be determined on dried and filtered fats and oils.

In the case of a solid sample, transfer the sample prepared in accordance with ISO 661 to a suitable container and place it in the water bath (6.5), set at the temperature at which the measurements are to be made. Allow sufficient time for the temperature of the sample to stabilize.

## 9 Procedure

**NOTE** If it is required to check whether the repeatability requirement (11.2) is met, carry out two single determinations in accordance with 9.1 and 9.2.

### 9.1 Calibration of the instrument

Verify the calibration of the refractometer (6.1) by measuring the refractive index of the glass plate (6.3) in accordance with the manufacturer's instructions, or by measuring the refractive index of the ethyl laurate (5.1).

### 9.2 Determination

Measure the refractive index of the test sample at one of the following temperatures:

- a) 20 °C for fats and oils that are completely liquid at this temperature;
- b) 40 °C for fats and oils that are completely melted at this temperature but not at 20 °C;
- c) 50 °C for fats and oils that are completely melted at this temperature but not at 40 °C;
- d) 60 °C for fats and oils that are completely melted at this temperature but not at 50 °C;
- e) 80 °C or above for other fats and oils, for example completely hardened fats or waxes.

Maintain the temperature of the prism of the refractometer at the required constant value by circulating through the instrument water from the water bath (6.4).

Monitor the temperature of the water issuing from the refractometer using a suitable precision thermometer. Immediately before the measurement, lower the movable part of the prism to a horizontal position. Wipe the surface of the prism with a soft cloth and then with a piece of cotton wool moistened with a few drops of the solvent (5.2). Allow to dry.

Carry out the measurement according to the operating instructions for the instrument being used. Read the refractive index to the nearest 0,000 1 as an absolute value, and record the temperature of the prism of the instrument.

Immediately after the measurement, wipe the surface of the prism with a soft cloth and then with a piece of cotton wool moistened with a few drops of the solvent (5.2). Allow to dry.

Measure the refractive index twice more, calculate the arithmetic mean of the three measurements and take this as the test result.

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## 10 Calculation

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If the difference between the measurement temperature  $t_1$  and the reference temperature  $t$  is less than 3 °C, the refractive index  $n_D^t$  at the reference temperature  $t$  is given by the following equation:

$$n_D^t = n_D^{t_1} + (t_1 - t)F$$

where

$t_1$  is the measurement temperature, in degrees Celsius;

$t$  is the reference temperature (see 9.2), in degrees Celsius;

$F$  is the factor equal to

0,000 35 at  $t = 20$  °C

0,000 36 at  $t = 40$  °C,  $t = 50$  °C and  $t = 60$  °C

0,000 37 at  $t = 80$  °C or above.

If the difference between the measurement temperature  $t_1$  and the reference temperature  $t$  is 3 °C or more, the result should be discarded and a fresh determination made.

Report the result rounded to the fourth decimal place.

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test on the precision method are summarized in annex A. The values derived from this test 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 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 not more than 5 % of cases be greater than the repeatability limit  $r$  given in annex A.

### 11.3 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, will in not more than 5 % of cases be greater than the reproducibility limit  $R$  given in annex A.

## 12 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known with reference to this International Standard;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result;
- the test result(s) obtained, or if the repeatability has been checked, the final result obtained.

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## Annex A (informative)

### Results of an interlaboratory test

A national collaborative test involving nine laboratories in Germany was carried out on:

- rapeseed oil (A),
- sunflower seed oil (B),
- modified linseed oil (C),
- modified castor oil (D),
- castor oil (E).

The statistical analysis was performed in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table A.1.

Table A.1 — Summary of statistical results

	Sample				
	A	B	C	D	E
Number of participating laboratories	9	9	9	9	9
Number of laboratories retained after eliminating outliers	9	9	9	9	9
Number of individual test results of all laboratories on each sample	45	45	45	45	45
Mean value	1,473 24	1,4575 12	1,482 33	1,483 91	1,479 30
Repeatability standard deviation, $s_r$	0,000 06	0,000 06	0,000 06	0,000 05	0,000 05
Repeatability coefficient of variation, %	0,01	0,01	0,01	0,01	0,01
Repeatability limit, $r$ ( $2,8 s_r$ )	0,000 17	0,000 17	0,000 17	0,000 15	0,000 13
Reproducibility standard deviation ( $s_R$ )	0,000 27	0,000 30	0,000 33	0,000 40	0,000 35
Reproducibility coefficient of variation, %	0,05	0,06	0,06	0,08	0,07
Reproducibility limit, $R$ ( $2,8 s_R$ )	0,000 75	0,000 84	0,000 94	0,001 12	0,000 98