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**Animal and vegetable fats and oils —  
Determination of acid value and acidity**

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

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# Contents

Page

Foreword.....	iv
<b>1 Scope.....</b>	<b>1</b>
<b>2 Normative references.....</b>	<b>1</b>
<b>3 Terms and definitions.....</b>	<b>1</b>
<b>4 Principle.....</b>	<b>2</b>
<b>5 Reagents.....</b>	<b>2</b>
<b>6 Apparatus.....</b>	<b>3</b>
<b>7 Sampling.....</b>	<b>3</b>
<b>8 Preparation of test sample.....</b>	<b>3</b>
<b>9 Procedure.....</b>	<b>3</b>
9.1 Cold solvent method using indicator (reference method).....	3
9.2 Cold solvent method using potentiometric titration (reference method).....	4
9.3 Hot ethanol method using indicator.....	4
<b>10 Calculation.....</b>	<b>5</b>
10.1 General.....	5
10.2 Acid value.....	5
10.3 Acidity or free fatty acid content.....	5
<b>11 Precision.....</b>	<b>6</b>
11.1 Repeatability.....	6
11.2 Reproducibility.....	6
<b>12 Test report.....</b>	<b>6</b>
<b>Annex A (informative) Results of interlaboratory tests.....</b>	<b>7</b>
<b>Annex B (informative) Comparison of three coloured indicators.....</b>	<b>10</b>
<b>Bibliography.....</b>	<b>12</b>

## 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 307, *Oilseeds, vegetable and animal fats and oils and their by-products — Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This fourth edition cancels and replaces the third edition (ISO 660:2009), which has been technically revised. The main changes compared with the previous edition are as follows:

- a non-applicability statement for milk and milk products has been added to the Scope because ISO 1740 applies in these cases;
- details of a CMR classification on coloured indicators have been added;
- data from the collaborative trial carried out to support this modification have been added in [Annex B](#).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

# Animal and vegetable fats and oils — Determination of acid value and acidity

## 1 Scope

This document specifies three methods (two titrimetric and one potentiometric) for the determination of acidity in animal and vegetable fats and oils, hereinafter referred to as “fats”. The acidity is expressed preferably as acid value or, alternatively, as acidity calculated conventionally.

This document is applicable to refined and crude vegetable or animal fats and oils, soap stock fatty acids or technical fatty acids. It does not apply to waxes.

Since the methods are completely non-specific, they do not apply to differentiating between mineral acids, free fatty acids and other organic acids. The acid value, therefore, includes any mineral acids that are present.

Milk and milk products (or fat coming from milk and milk products) are excluded from the Scope of this document.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 385, *Laboratory glassware — Burettes*

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

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

## 3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

### 3.1

#### acid value

number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of fat, when determined in accordance with the procedure specified in this document

Note 1 to entry: The acid value is expressed in milligrams per gram.

### 3.2

#### acidity

content of free fatty acids determined according to the procedure specified in this document

Note 1 to entry: The acidity is expressed as a percentage by mass. If the result of the determination is reported as acidity without further explanation, this is, by convention, the acidity based on the oleic acid content.

## 4 Principle

The sample is dissolved in a suitable solvent mixture, and the acids present are titrated with an ethanolic or methanolic solution of sodium or potassium hydroxide.

The methods specified in [9.1](#) and [9.2](#) are reference methods.

## 5 Reagents

**WARNING** — Attention is drawn to regulations that specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.

Use only reagents of recognized analytical grade, unless otherwise specified.

**5.1 Solvent A for the solvent mixture** ([5.3](#)), ethanol, volume fraction,  $\varphi \approx 96\%$ .

As a replacement, propan-2-ol, volume fraction,  $\varphi \approx 99\%$ , may be used.

**5.2 Solvent B for the solvent mixture** ([5.3](#)), diethyl ether, peroxide-free.

As a replacement, *tert*-butyl methyl ether, light petroleum (boiling range 40 °C to 60 °C) or toluene may be used.

**WARNING** — Diethyl ether is very flammable and may form explosive peroxides. Use with great caution.

**5.3 Solvent mixture**, mix equal volumes of solvents A ([5.1](#)) and B ([5.2](#)). Volume fraction of A:  $\varphi = 50\text{ ml}/100\text{ ml}$ ; volume fraction of B:  $\varphi = 50\text{ ml}/100\text{ ml}$ .

For hard or animal fats, a solvent mixture of one volume of solvent A (e.g. 25 ml) and three volumes of *tert*-butyl methyl ether or toluene (e.g. 75 ml) is recommended.

Neutralize, just before use, by adding potassium hydroxide solution in the presence of 0,3 ml of the coloured indicator solution per 100 ml of solvent mixture.

For the titration with aqueous KOH, the solvent propan-2-ol may be used.

**5.4 Ethanol or methanol**, of minimum volume fraction,  $\varphi = 95\%$ .

**5.5 Sodium hydroxide or potassium hydroxide standard volumetric solution**, made up in either ethanol or methanol. Substance concentration  $c(\text{NaOH})$  or  $c(\text{KOH})$ : 0,1 mol/l and 0,5 mol/l, respectively. The exact concentration of the sodium hydroxide solution (or potassium hydroxide solution) shall be known or checked prior to use.

**NOTE** The ethanolic/methanolic sodium/potassium hydroxide solution can be replaced by an aqueous sodium/potassium hydroxide solution, but only if the volume of water introduced does not lead to phase separation.

**5.6 Thymolphthalein** (CAS number: 125-20-2) or **Alkali blue 6B** (CAS number: 1324-76-1) solution in ethanol, mass concentration,  $\rho = 2\text{ g}/100\text{ ml}$  or, failing that, **Phenolphthalein** (CAS number: 77-09-8) solution in ethanol, mass concentration,  $\rho = 1\text{ g}/100\text{ ml}$ .

**NOTE 1** Phenolphthalein is classified as a carcinogenic, mutagenic or toxic for reproduction (CMR) substance whereas thymolphthalein and alkali blue are not.

**NOTE 2** A laboratory test has been done in order to compare the three colour indicators (see [Annex B](#)).

For dark-coloured fats, **alkali blue** or **thymolphthalein** shall be used.

Phenolphthalein is a known carcinogenic compound and should only be used when no alternative is available.

**5.7 Water**, in accordance with ISO 3696, grade 3.

## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

**6.1 Burette**, capacity 10 ml, graduated in 0,02 ml, ISO 385, class A.

**6.2 Burette**, capacity 25 ml, graduated in 0,05 ml, ISO 385, class A.

**6.3 Analytical balance**, capable of being read to the nearest 0,001 g.

**6.4 Automatic titration apparatus** (based on potentiometric electrode) or potentiometer.

**6.5 Combined pH electrode** for non-aqueous acid/base titrations.

**6.6 Graduated volumetric flasks**, volume 1 000 ml, ISO 1042, class A.

## 7 Sampling

A representative sample should be sent to the laboratory. It should not be damaged or changed during transport or storage.

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

## 8 Preparation of test sample

Prepare the test sample in accordance with ISO 661. If the sample contains volatile fatty acids, the test sample shall not be heated and filtered.

## 9 Procedure

### 9.1 Cold solvent method using indicator (reference method)

**9.1.1** Depending on the expected magnitude of the acid value, select the test portion mass and alkali concentration from [Table 1](#).

**9.1.2** According to [Table 1](#), weigh the test portion into a 250 ml conical flask.

**9.1.3** Add 50 ml to 100 ml of the neutralized solvent mixture ([5.3](#)) and dissolve the test portion, if necessary, with gentle warming.

For high melting point samples, use an ethanol-toluene mixture.

9.1.4 After the addition of an indicator (5.6), titrate with constant swirling using sodium hydroxide or potassium hydroxide solution (5.5). The endpoint of the titration is reached when the addition of a single drop of alkali produces a slight but definite colour change persisting for at least 15 s.

**Table 1 — Test portion masses and alkali (NaOH or KOH) concentrations**

Product group (examples)	Acid value approx.	Mass of test portion	Concentration of NaOH or KOH	Accuracy of weighing of the test portion
		g	mol/l	g
Refined vegetable oils Animal fats	0 to 1	20	0,1	0,05
Crude vegetable oils Technical grade animal fats	1 to 4 4 to 15	10 2,5	0,1 0,1	0,02 0,01
Soap stock fatty acids	15 to 75	0,5	0,1	0,001
		3,0	0,5	
Technical fatty acids	> 75	0,2	0,1	0,001
		1,0	0,5	

## 9.2 Cold solvent method using potentiometric titration (reference method)

9.2.1 According to Table 1, weigh the test portion into a 150 ml beaker.

9.2.2 Add 50 ml to 100 ml of the neutralized solvent mixture (5.3) and dissolve the sample, if necessary, with gentle warming.

For high melting point samples, use an ethanol-toluene mixture.

9.2.3 Introduce the combined electrode in the solvent mixture and connect it with the automatic titration apparatus.

9.2.4 Start the stirrer for at least 30 s and then titrate with constant swirling using sodium hydroxide or potassium hydroxide solution (5.5).

9.2.5 As soon as the equivalence point is reached, record the amount of standard solution used.

## 9.3 Hot ethanol method using indicator

9.3.1 Under the conditions specified in this method, short-chain fatty acids, if present, are volatile.

9.3.2 Weigh into a flask a sufficient mass of the test sample as shown in Table 1, according to the colour and expected acid value.

9.3.3 Heat to boiling 50 ml of the ethanol containing 0,5 ml of the indicator (5.6) in a second flask. While the temperature of the ethanol is still above 70 °C, neutralize it carefully with a solution of 0,1 mol/l sodium or potassium hydroxide.

The endpoint of the titration is reached when the addition of a single drop of alkali produces a slight but definite colour change persisting for at least 15 s.

Larger volumes of ethanol and indicator may be necessary for dark-coloured fats. Moreover, for dark-coloured fats, alkali blue or thymolphthalein shall be used.

**9.3.4** Add the neutralized ethanol to the test portion in the first flask and mix thoroughly. Bring the contents to the boil and titrate with the sodium or potassium hydroxide solution, agitating the flask contents vigorously during the titration.

## 10 Calculation

### 10.1 General

The acid value,  $w_{AV}$ , or the free fatty acid content,  $w_{FFA}$ , is reported as follows:

- to two decimal places for values between 0 up to and including 1;
- to one decimal place for values between 1 up to and including 100;
- as a whole number for values  $> 100$ .

In addition to the following calculations, the approximate acidity or free fatty acid content is calculated from [Formula \(1\)](#):

$$w_{FFA} = 0,5 \times w_{AV} \quad (1)$$

### 10.2 Acid value

The acid value,  $w_{AV}$ , expressed as a mass fraction, is equal to [Formula \(2\)](#):

$$w_{AV} = \frac{56,1 \times cV}{m} \quad (2)$$

where

- $c$  is the exact concentration, in moles per litre, of the standard volumetric sodium or potassium hydroxide solution used;
- $V$  is the volume, in millilitres, of standard volumetric sodium or potassium hydroxide solution used;
- $m$  is the mass, in grams, of the test portion.

### 10.3 Acidity or free fatty acid content

The acidity or free fatty acid content,  $w_{FFA}$ , expressed as a percentage mass fraction, and according to fat type (see [Table 2](#)), is equal to [Formula \(3\)](#):

$$w_{FFA} = \frac{VcM \times 100}{1000 \times m} \quad (3)$$

where

- $V$  is the volume, in millilitres, of the standard volumetric sodium or potassium hydroxide solution used;
- $c$  is the concentration, in moles per litre, of the standard volumetric sodium or potassium hydroxide solution used;
- $M$  is the molar mass, in grams per mole, of the acid chosen for expression of the result (see [Table 2](#)) according to the fat type;
- $m$  is the mass, in grams, of the test portion.

Table 2 — Choice of fatty acid for expression of acidity

Type of fat	Expressed as	Molar mass g/mol
Coconut oil Palm kernel oil and similar oils	Lauric acid	200
Palm oil	Palmitic acid	256
Oils from certain <i>Cruciferae</i> <sup>a</sup>	Erucic acid	338
All other fats	Oleic acid	282

<sup>a</sup> In the case of rapeseed oil having a maximum erucic acid content of 5 %, the acidity shall be expressed as oleic acid.

NOTE If the result is reported simply as “acidity”, without further definition, this is, by convention, expressed as oleic acid. If the sample contains mineral acids, these are, by convention, determined as fatty acids.

## 11 Precision

Details of interlaboratory tests are given in [Annex A](#). The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

### 11.1 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, shall in not more than 5 % of cases exceed the values given in [Tables A.1](#) to [A.3](#).

### 11.2 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, shall in not more than 5 % of cases exceed the values given in [Tables A.1](#) to [A.3](#).

## 12 Test report

The test report shall contain at least the following information:

- all information necessary for the complete identification of the sample;
- a reference to this document, i.e. ISO 660;
- the method used: “cold solvent method using indicator” or “cold solvent using potentiometric titration” or “hot ethanol method using indicator method”;
- the result obtained, indicating clearly the method of expression used;
- any operating conditions not specified in this document or regarded as optional;
- the date of the test.

## Annex A (informative)

### Results of interlaboratory tests

The precision of the method is the result of interlaboratory studies on an international basis. The results are given in [Table A.1](#) for the reference methods in [9.1](#) and [9.2](#), and in [Tables A.2](#) and [A.3](#) for the hot ethanol method in [9.3](#).

A series of interlaboratory tests, carried out by a different number of laboratories, using the methods described in [9.1](#) to [9.3](#), gave the statistical results [evaluated in accordance with ISO 5725:1986 and ISO 5725 (all parts)] given in [Tables A.1](#) to [A.3](#).

**Table A.1 — Summary of statistical results (acid value, expressed as mg KOH/g fat)**

Sample	Refined rapeseed oil	Lard	Crude sunflower seed oil	Lampante virgin olive oil	Cold pressed wheat germ oil	Technical fatty acids
Number of participating laboratories, $N$	26	26	26	26	26	26
Number of laboratories retained after eliminating outliers, $n$	25	24	26	24	23	24
Number of individual test results of all laboratories on each sample, $n_z$	50	48	52	48	46	48
<b>Mean value, <math>\bar{w}_{AV}</math>, in mg/g<sup>a</sup></b>	<b>0,080</b>	<b>0,381</b>	<b>1,39</b>	<b>5,48</b>	<b>7,48</b>	<b>128,1</b>
Repeatability standard deviation, $s_r$ , in mg/g <sup>a</sup>	0,003	0,006	0,04	0,07	0,08	0,6
Repeatability coefficient of variation, $C_{V,r}$ , in %	3,6	1,7	2,6	1,2	1,1	0,4
<b>Repeatability limit, <math>r</math> (<math>s_r \times 2,8</math>), in mg/g<sup>a</sup></b>	<b>0,008</b>	<b>0,018</b>	<b>0,10</b>	<b>0,19</b>	<b>0,23</b>	<b>1,6</b>
Reproducibility standard deviation, $s_R$ , in mg/g <sup>a</sup>	0,018	0,019	0,05	0,15	0,40	2,6
Reproducibility coefficient of variation, $C_{V,R}$ , in %	22,2	5,0	3,6	2,7	5,3	2,1
<b>Reproducibility limit, <math>R</math> (<math>s_R \times 2,8</math>), in mg/g<sup>a</sup></b>	<b>0,049</b>	<b>0,053</b>	<b>0,14</b>	<b>0,41</b>	<b>1,12</b>	<b>7,4</b>
<sup>a</sup> The precision data for the acidity as a percentage of free fatty acids can be calculated by dividing the corresponding values for the acid value by 1,99.						

**Table A.2 — Summary of statistical results (acidity, expressed as a percentage mass fraction)**

Sample	Extra virgin olive oil	Lampante virgin olive oil		Olive oil	Sunflower seed oil	Coconut oil
Number of participating laboratories, $N$	39	28	28	37	23	25
Number of laboratories retained after eliminating outliers, $n$	37	26	24	37	22	25
Number of individual test results of all laboratories on each sample, $n_z$	74	52	48	74	44	50
<b>Mean value, <math>\bar{w}_{\text{FFA}}</math>, in % mass fraction</b>	<b>0,343</b>	<b>3,80</b>	<b>19,55</b>	<b>0,604</b>	<b>0,830</b>	<b>1,49</b>
Repeatability standard deviation, $s_r$ , in %	0,007	0,03	0,09	0,012	0,009	0,009
Repeatability coefficient of variation, $C_{V,r}$ , in %	1,9	0,8	0,5	1,9	1,1	0,6
<b>Repeatability limit, <math>r</math></b> ( $s_r \times 2,8$ ), in %	<b>0,018</b>	<b>0,07</b>	<b>0,26</b>	<b>0,033</b>	<b>0,025</b>	<b>0,025</b>
Reproducibility standard deviation, $s_R$ , in %	0,019	0,12	0,60	0,035	0,027	0,027
Reproducibility coefficient of variation, $C_{V,R}$ , in %	5,5	3,2	3,1	5,8	3,3	1,8
<b>Reproducibility limit, <math>R</math></b> ( $s_R \times 2,8$ ), in %	<b>0,053</b>	<b>0,33</b>	<b>1,67</b>	<b>0,098</b>	<b>0,075</b>	<b>0,075</b>

**Table A.3 — Summary of statistical results (acidity, expressed as a percentage mass fraction)**

Sample	Palm oil		Palm kernel fat		
Number of participating laboratories, $N$	12	27	41	41	23
Number of laboratories retained after eliminating outliers, $n$	10	27	39	40	22
Number of individual test results of all laboratories on each sample, $n_z$	20	54	78	80	44
<b>Mean value, <math>\bar{w}_{\text{FFA}}</math>, in % mass fraction</b>	<b>3,11</b>	<b>4,09</b>	<b>6,46</b>	<b>1,72</b>	<b>7,26</b>
Repeatability standard deviation, $s_r$ , in %	0,01	0,021	0,03	0,02	0,03
Repeatability coefficient of variation, $C_{V,r}$ , in %	0,4	0,5	0,4	0,1	0,3
<b>Repeatability limit, <math>r</math></b> ( $s_r \times 2,8$ ), in %	<b>0,03</b>	<b>0,06</b>	<b>0,07</b>	<b>0,06</b>	<b>0,07</b>
Reproducibility standard deviation, $s_R$ , in %	0,161	0,064	0,082	0,07	0,085
Reproducibility coefficient of variation, $C_{V,R}$ , in %	5,2	1,6	1,3	4,1	1,2
<b>Reproducibility limit, <math>R</math></b> ( $s_R \times 2,8$ ), in %	<b>0,45</b>	<b>0,18</b>	<b>0,23</b>	<b>0,20</b>	<b>0,24</b>

## Annex B (informative)

### Comparison of three coloured indicators

A series of interlaboratory tests, carried out with the three coloured indicators proposed in 9.1, gave the statistical results given in Tables B.1 to B.3.

The statistic study did not show any significant difference between these coloured indicators.

**Table B.1 — Summary of statistical results — Refined sunflower oil (acidity, expressed as a percentage mass fraction)**

Coloured indicator	Alkali Blue 6B	Thymolphthalein	Phenolphthalein
Number of individual test results of all laboratories on each sample, $n_z$	12	12	12
<b>Mean value, <math>\bar{w}_{\text{FFA}}</math>, in % mass fraction</b>	<b>0,01</b>	<b>0,02</b>	<b>0,02</b>
Repeatability standard deviation, $s_r$ , in %	0,000	0,004	0,008
Repeatability coefficient of variation, $C_{V,r}$ , in %	0,00	19,65	39,31
<b>Repeatability limit, <math>r</math></b> ( $s_r \times 2,8$ ), in %	<b>0,000</b>	<b>0,011</b>	<b>0,022</b>
Reproducibility standard deviation, $s_R$ , in %	0,009	0,011	0,010
Reproducibility coefficient of variation, $C_{V,R}$ , in %	61,75	57,05	52,75
<b>Reproducibility limit, <math>R</math></b> ( $s_R \times 2,8$ ), in %	<b>0,024</b>	<b>0,031</b>	<b>0,029</b>

**Table B.2 — Summary of statistical results — Hazelnut oil (acidity, expressed as a percentage mass fraction)**

Coloured indicator	Alkali Blue 6B	Thymolphthalein	Phenolphthalein
Number of individual test results of all laboratories on each sample, $n_z$	12	12	12
<b>Mean value, <math>\bar{w}_{\text{FFA}}</math>, in % mass fraction</b>	<b>0,72</b>	<b>0,77</b>	<b>0,76</b>
Repeatability standard deviation, $s_r$ , in %	0,011	0,012	0,017
Repeatability coefficient of variation, $C_{V,r}$ , in %	1,53	1,50	2,28
<b>Repeatability limit, <math>r</math></b> ( $s_r \times 2,8$ ), in %	<b>0,031</b>	<b>0,032</b>	<b>0,048</b>
Reproducibility standard deviation, $s_R$ , in %	0,029	0,023	0,035
Reproducibility coefficient of variation, $C_{V,R}$ , in %	3,98	3,03	4,58
<b>Reproducibility limit, <math>R</math></b> ( $s_R \times 2,8$ ), in %	<b>0,08</b>	<b>0,065</b>	<b>0,097</b>