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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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Foreword

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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 660 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This third edition cancels and replaces the second edition (ISO 660:1996), which has been technically revised. It also incorporates the Amendment ISO 660:1996/Amd 1:2003.

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

1 Scope

This International Standard specifies three methods (two titrimetric and one potentiometric) for the determination of the 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 International Standard is applicable to refined and crude vegetable or animal fats and oils, soap stock fatty acids or technical fatty acids. The methods are not applicable to waxes.

Since the methods are completely non-specific, they cannot be used to differentiate between mineral acids, free fatty acids, and other organic acids. The acid value, therefore, also includes any mineral acids that may be present.

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 applies.

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

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.

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 International Standard

NOTE 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 International Standard

NOTE 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 potassium or sodium hydroxide.

The methods specified in 9.1 and 9.2 are reference methods.

5 Reagents

WARNING — Attention is drawn to the regulations which 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 solvent mixture (5.3): ethanol, volume fraction, $\varphi \approx 96$ %.

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

5.2 Solvent B for 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 can 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 solvent A and B, (e.g. $\varphi_A = 50$ ml/100 ml and $\varphi_B = 50$ ml/100 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 phenolphthalein solution per 100 ml of solvent mixture.

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

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

5.5 Sodium hydroxide or **potassium hydroxide**, ethanolic or methanolic standard volumetric solutions, amount of substance concentration $c(\text{NaOH})$ or $c(\text{KOH}) = 0{,}1$ mol/l and 0,5 mol/l. The concentration shall be checked with a standard volumetric HCl solution.

NOTE The ethanolic/methanolic sodium/potassium hydroxide solution may 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 Phenolphthalein, solution in ethanol, mass concentration, $\rho = 1$ g/100 ml.

5.7 Thymolphthalein, solution in ethanol, mass concentration, $\rho = 2$ g/100 ml.

5.8 Alkali blue 6B, solution in ethanol, mass concentration, $\rho = 2$ g/100 ml.

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

5.9 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[1] class A.

6.2 **Burette**, capacity 25 ml, graduated in 0,05 ml, ISO 385[1] 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[2] class A.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have 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 5555 [3].

8 Preparation of test sample

Prepare the test sample in accordance with ISO 661, except that 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, 5.7 or 5.8), titrate with constant swirling using standard 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 concentrations

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

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 standard 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 phenolphthalein indicator 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

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

a) to two decimal places for values between 0 up to and including 1;

b) to one decimal place for values between 1 up to and including 100;

c) as a whole number for values > 100.

In addition to the following calculations, the approximate free fatty acid content (acidity) is calculated from:

$$w_{FFA} = 0{,}5 \times w_{AV}$$

10.1 Acid value

The acid value, w_{AV}, expressed as a mass fraction, is equal to

$$w_{AV} = \frac{56{,}1 \times c\, V}{m}$$

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.

10.2 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

$$w_{FFA} = \frac{V\, c\, M \times 100}{1\,000 \times m}$$

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 *Cruciferae* [a]	Erucic acid	338
All other fats	Oleic acid	282
[a] 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:

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

b) a reference to this International Standard;

c) the result obtained, indicating clearly the method of expression used;

d) any operating conditions not specified in this International Standard, or regarded as optional.

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 (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[4] and ISO 5725 (all parts)[5]] 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
Mean value, $\bar{w}_{AV}$, mg/g [a]	**0,080**	**0,381**	**1,39**	**5,48**	**7,48**	**128,1**
Repeatability standard deviation, s_r, mg/g [a]	0,003	0,006	0,04	0,07	0,08	0,6
Repeatability coefficient of variation, CV(r), %	3,6	1,7	2,6	1,2	1,1	0,4
Repeatability limit, r ($s_r \times 2{,}8$), mg/g [a]	**0,008**	**0,018**	**0,10**	**0,19**	**0,23**	**1,6**
Reproducibility standard deviation, s_R, mg/g [a]	0,018	0,019	0,05	0,15	0,40	2,6
Reproducibility coefficient of variation, CV(R), %	22,2	5,0	3,6	2,7	5,3	2,1
Reproducibility limit, R ($s_R \times 2{,}8$), mg/g [a]	**0,049**	**0,053**	**0,14**	**0,41**	**1,12**	**7,4**

[a] 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.