INTERNATIONAL STANDARD

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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

INTERNATIONAL ISO

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

This second edition cancels and replaces the first edition (ISO 660:1983), which has been technically revised.

Annexes A and B of this International Standard are for information only.

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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 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.

The method described in clause 4 is the reference method. The method described in clause 5 applies to oils and fats which are not strongly coloured.

The methods are not applicable to waxes.

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 661:1989, *Animal and vegetable fats and oils — Preparation of test sample.*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

3 Definitions

For the purposes of this International Standard, the following 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.

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.

Acidity is expressed as a percentage by mass.

NOTES

1 If the result of the determination is reported as acidity, without further explanation, this is by convention the acidity expressed based on oleic acid.

2 If the sample contains mineral acids, these are, by convention, determined as fatty acids.

4 Hot ethanol method using indicator

4.1 General

This method is the reference method for fats (see clause 1).

NOTE 3 Under the conditions specified in this method, short-chain fatty acids, if present, are volatile.

4.2 Principle

A test portion is dissolved in hot ethanol and titrated with an aqueous solution of sodium or potassium hydroxide.

4.3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and water in accordance with grade 3 of ISO 3696.

4.3.1 Ethanol, of minimum purity 95 % (*V*/*V*).

4.3.2 Sodium or potassium hydroxide, standard volumetric solution, c(NaOH) or c(KOH) = 0,1 mol/l.

4.3.3 Sodium or potassium hydroxide, standard volumetric solution, c(NaOH) or c(KOH) = 0,5 mol/l.

4.3.4 Phenolphthalein, 10 g/l solution in ethanol [95 % (*V*/*V*)].

NOTE 4 In determinations of strongly coloured solutions, observation of the endpoint of the titration may be facilitated by adding 1 ml of a 0,1 % (*m*/*m*) solution of methylene blue to each 100 ml of phenolphthalein indicator solution.

4.3.5 Alkali blue 6B, or (in the case of dark-coloured fats) **thymolphthalein,** 20 g/l solution in ethanol, [95 % (*V*/*V*)].

4.4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.4.1 Microburette, of 10 ml capacity, graduated in 0,02 ml subdivisions.

4.4.2 Analytical balance, capable of weighing to the required accuracy (see table 1).

4.5 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 5555.

4.6 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.

4.7 Procedure

4.7.1 Test portion

Weigh into a flask a sufficient mass of the test sample (4.6) as shown in table 1, according to the colour and expected acid value.

NOTE 5 The mass of the test portion and the concentration of the titrant should be such that the titrate does not exceed 10 ml.

Table 1 — Mass of test portion

Expected acid value	Mass of test portion g	Accuracy of weighing of the test portion g
< 1	20	0,05
1 to 4	10	0,02
4 to 15	2,5	0,01
15 to 75	0,5	0,001
> 75	0,1	0,000 2

4.7.2 Determination

Heat to boiling 50 ml of the ethanol (4.3.1) containing 0,5 ml of the phenolphthalein indicator (4.3.4) in a second flask. Whilst the temperature of the ethanol is still over 70 °C, neutralize it carefully with a solution of 0,1 mol/l sodium or potassium hydroxide (4.3.2).

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.

NOTE 6 Larger volumes of ethanol and indicator may be necessary for dark-coloured fats.

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 (4.3.2 or 4.3.3, depending on the expected acidity of the sample), agitating the flask contents vigorously during the titration.

5 Cold solvent method using indicator

5.1 General

This method is most suited to fats which are not strongly coloured.

5.2 Principle

A test portion is dissolved in a mixed solvent and titrated with an ethanolic solution of potassium hydroxide.

5.3 Reagents

Use only reagents of recognized analytical grade, and water of grade 3 in accordance with ISO 3696.

5.3.1 Diethyl ether and 95 % (*V*/*V*) **ethanol**, 1+1 mixture by volume.

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

Neutralize, just before use, by adding the potassium hydroxide solution (5.3.2) in the presence of 0,3 ml of the phenolphthalein solution (5.3.3) per 100 ml of solvent mixture (see note 6 in 4.7.2).

If it is not possible to use diethyl ether, a mixed solvent may be used, as follows:

toluene and 95 % (*V*/*V*) ethanol, 1+1 mixture by volume,

toluene and 99 % (*V*/*V*) propan-2-ol, 1+1 mixture by volume.

The mixed solvent may be replaced by 99 % (*V*/*V*) propan-2-ol for both crude and refined vegetable fats.

5.3.2 Potassium hydroxide, standard volumetric solution in ethanol:

c(KOH) = 0,1 mol/l (solution A) or, if necessary

c(KOH) = 0,5 mol/l (solution B).

The ethanolic potassium hydroxide solution may be replaced by an aqueous potassium or sodium hydroxide solution, but only if the volume of water introduced does not lead to phase separation.

5.3.2.1 Dissolve 7 g (for solution A) or 35 g (for solution B) of potassium hydroxide pellets in ethanol (4.3.1) and dilute to 1 000 ml with the ethanol.

NOTE 7 Propan-2-ol may be used instead of ethanol.

Determine the concentration of the solution, immediately before use, as follows.

Weigh, to the nearest 0,000 2 g, either 0,15 g (for solution A) or 0,75 g (for solution B) of benzoic acid of minimum purity 99,9 % (*m*/*m*), or another primary standard, in a 150 ml beaker and dissolve in 50 ml of the 4-methylpentan-2-one (6.2.1).

Introduce the electrodes of a pH-meter (see 6.3.1), start the stirrer (6.3.2), and titrate with the potassium hydroxide solution (A or B, depending on the expected acidity of the sample) to the equivalence point (see 6.6.2.2).

The concentration of the potassium hydroxide solution, expressed in moles per litre, is given by:

$$\frac{1\,000 \cdot m_0}{122{,}1 \cdot V_0}$$

where

m_0 is the mass, in grams, of benzoic acid used;

V_0 is the volume, in millilitres, of potassium hydroxide solution used.

NOTE 8 In the above and later mathematical expressions, the symbols represent the numerical values of the quantities.

Use solution prepared at least 5 days previously and decanted into a brown glass bottle, fitted with a rubber stopper provided with a thermometer needed for temperature correction (see 7.1). The solution shall be colourless or straw yellow. If the bottle is connected to the burette, provision shall be made to prevent intake of carbon dioxide, for example by using a tube filled with granular soda lime.

5.3.2.2 A stable colourless solution of potassium hydroxide may also be prepared in the following manner. Boil, under reflux, 1 000 ml of ethanol with 8 g of potassium hydroxide and 0,5 g of aluminium pellets for 1 h, then distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow the whole to stand for several days and decant the clear supernatant liquid from the deposited potassium carbonate.

5.3.2.3 The solution may also be prepared without distillation in the following manner. Add 4 ml of aluminium tri-*sec*-butoxide to 1 000 ml of ethanol and allow the mixture to stand for several days. Decant the supernatant liquid and dissolve in it the required amount of potassium hydroxide. This solution is ready for use.

5.3.3 Phenolphthalein, see 4.3.4.

5.3.4 Alkali blue 6B or **thymolphthalein,** see 4.3.5.

5.4 Apparatus

Usual laboratory equipment and, in particular, the **microburette** described in 4.4.1.

5.5 Sampling

See 4.5.

5.6 Preparation of test sample

See 4.6.

5.7 Procedure

5.7.1 Test portion

Take a sufficient mass of the test sample (5.6), according to the expected acid value, in accordance with table 1.

Weigh the test portion into a 250 ml conical flask.

5.7.2 Determination

5.7.2.1 Dissolve the test portion (5.7.1) in 50 ml to 150 ml of the previously neutralized solvent mixture (5.3.1).

Titrate, whilst swirling the solution, with the potassium hydroxide solution (5.3.2) (see 5.7.2.3) to the endpoint as described in 4.7.2.

5.7.2.2 In the case of very low acid values (< 1), it is preferable to pass a gentle flow of nitrogen through the test solution.

5.7.2.3 If the quantity of potassium hydroxide solution A (0,1 mol/l) required exceeds 10 ml, use solution B (0,5 mol/l).

5.7.2.4 If the solution becomes turbid during titration, add a sufficient quantity of the mixed solvent (5.3.1) to give a clear solution.

6 Potentiometric method

6.1 Principle

Potentiometric titration of the three fatty acids in a test portion with a solution of potassium hydroxide and propan-2-ol in a non-aqueous medium.

6.2 Reagents

Use only reagents of recognized analytical grade and water in accordance with grade 3 of ISO 3696.

6.2.1 4-Methylpentan-2-one (previously known as methyl isobutyl ketone), neutralized just before use by adding the potassium hydroxide solution (6.2.2), using the pH-meter (6.3.1).

6.2.2 Potassium hydroxide, standard volumetric solution C, c(KOH) = 0,1 mol/l, in propan-2-ol, prepared and standardized as described in 5.3.2 for solution A.

6.2.3 Potassium hydroxide, standard volumetric solution D, c(KOH) = 0,5 mol/l, in propan-2-ol, prepared and standardized as described in 5.3.2 for solution B.

6.3 Apparatus

Use laboratory apparatus and, in particular, the following.

6.3.1 pH-meter, equipped with glass and calomel electrodes.

Contact between the saturated potassium chloride solution and the test solution shall be made across a sintered glass or porcelain plate at least 3 mm thick.

NOTES

9 It is advisable to store the glass electrode for 12 h before titration in the 4-methylpentan-2-one (6.2.1). Dry it very gently with a filter paper before making the measurement. Rinse it immediately after the determination with 4-methylpentan-2-one, then with propan-2-ol and finally with distilled water.

10 If the electrode does not function satisfactorily, try to regenerate it by keeping it for 14 h in a 1 mol/l solution of isopropanolic hydrochloric acid. After this treatment, wash the electrode with distilled water, then with propan-2-ol and 4-methylpentan-2-one

11 The use of porcelain or thick glass plates to ensure contact between the saturated potassium chloride solution and the test solution prevents diffusion currents and accidental potential differences.

6.3.2 Stirrer, preferably a magnetic stirrer.

6.3.3 Analytical balance, as in 4.4.2.

6.4 Sampling

See 4.5.

6.5 Preparation of sample

See 4.6.

6.6 Procedure

6.6.1 Test portion

Weigh, to the nearest 0,01 g, 5 g to 10 g of the test sample (6.5) into a tall-form 150 ml beaker.

6.6.2 Determination

6.6.2.1 Dissolve the test portion (6.6.1) in 50 ml of the 4-methylpentan-2-one (6.2.1).

Introduce the electrodes of the pH-meter (6.3.1), start the stirrer (6.3.2) and titrate with the potassium hydroxide solution (6.2.2 or 6.2.3, depending on the expected acidity of the sample) to the equivalence point.

NOTE 12 The equivalence point is generally near to the value on the pH scale, and can be determined graphically by observing the inflection point on the neutralization curve. It can also be calculated by taking the value for which the first differential of the variation of pH (as a function of the volume of potassium hydroxide solution added) reaches a maximum, or the value for which the second differential becomes zero.

6.6.2.2 It is not possible to determine the inflection point in the case of crude cottonseed oils rich in gossypol. In this case, use a conventional determination of the inflection point, fixed arbitrarily at the pH of the equivalence point of the neutralization of oleic acid by potassium hydroxide in the solvent used for the titration, as follows.

Dissolve approximately 0,282 g of oleic acid in 50 ml of the 4-methylpentan-2-one (6.2.1). Plot the neutralization curve of the oleic acid by the potassium hydroxide solution (6.2.2 or 6.2.3) to be used. Read from the curve the pH of the inflection point (corresponding, in principle, to the addition of 10 ml of 0,1 mol/l potassium hydroxide solution). Using this value, read from the neutralization curve of the cottonseed oil the amount of potassium hydroxide solution used to "neutralize" the cottonseed oil.

7 Calculation

7.1 Acid value

The acid value is equal to

$$\frac{56{,}1 \cdot V \cdot c}{m}$$

where

V is the volume, in millilitres, of standard volumetric sodium or potassium hydroxide solution used;

c is the exact concentration, in moles per litre, of the standard volumetric sodium or potassium hydroxide solution used;

m is the mass, in grams, of the test portion.

NOTE 13 The concentration of the ethanolic sodium or potassium hydroxide solution varies with temperature and it may be useful to use the following correction:

$$V' = V_t \left[1 - 0{,}0011(t - t_0)\right]$$

where

V' is the corrected volume, in millilitres, of the standard sodium or potassium hydroxide solution;

V_t is the volume, in millilitres, of the standard sodium or potassium hydroxide solution measured at temperature t;

t is the temperature at which the determination was carried out, in degrees Celsius;

t_0 is the temperature, in degrees Celsius, at which the concentration of the standard sodium or potassium hydroxide solution was determined.

7.2 Acidity

The acidity, expressed as a percentage by mass, and according to fat type (see table 2), is equal to

$$V \cdot c \cdot \frac{M}{1\,000} \cdot \frac{100}{m} = \frac{V \cdot c \cdot M}{10 \cdot m}$$

where

V is the volume, in millilitres, of the standard volumetric sodium or potassium hydroxide solution used (see note 13 in 7.1);

c is the exact 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);

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*[1)]	Erucic acid	338
All other fats	Oleic acid	282

1) In the case of rapeseed oil having a maximum erucic acid content of 5 % (*m/m*), the acidity shall be expressed as oleic acid.

NOTES

14 If the result is reported simply as "acidity", without further definition, this is, by convention, expressed as oleic acid.

15 If the sample contains mineral acids, these are, by convention, determined as fatty acids.

8 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.

8.1 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

3 % of the mean of the two results when the acidity is 3 % or less;

1 % of the mean of the two results when the acidity is greater than 3 %.

8.2 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

15 % of the mean of the two results when the acidity is 3 % or less;

5 % of the mean of the two results when the acidity is greater than 3 %.

9 Test report

The test report shall specify:

— the method in accordance with which sampling was carried out, if known,

— the method used,

— the result obtained, indicating clearly the method of expression used and,

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any incidents that may have influenced the result.

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

Annex A
(informative)

Results of interlaboratory tests

A series of interlaboratory tests, carried out at the international level using the reference method described in clause 4, with each laboratory carrying out two determinations of each laboratory sample, gave the statistical results (evaluated in accordance with ISO 5725) shown below. For the repeatability and reproducibility limits obtained, a probability of 95 % holds.

Oil	Palm kernel	Sunflower	Coconut	Palm oil	Palm oil	Palm kernel	Palm kernel
Number of laboratories	23	23	25	12	27	41	41
Number of acceptable results	22	22	25	10/11	27	39	40
Mean acidity values, %	7,26	0,83	1,49	3,11	4,09	6,46	1,72
Repeatability, r	0,07	0,025	0,025	0,03	0,06	0,07	0,06
Reproducibility, R	0,24	0,075	0,075	0,45	0,18	0,23	0,20