INTERNATIONAL STANDARD

ISO 3657

Fifth edition
2020-04

Animal and vegetable fats and oils — Determination of saponification value

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

Reference number
ISO 3657:2020(E)

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Published in Switzerland

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Foreword

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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).

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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.

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 fifth edition cancels and replaces the fourth edition (ISO 3657:2013), of which it constitutes a minor revision. The changes compared with the previous edition are as follows:

— corrects the mistake in the calculation of the C16 TAG molecular weight (B.7.3);

— stipulates using the preferred indicator, alkali blue, to phenolphthalein (5.4 and 5.5) for safety reasons;

— updates the references.

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.

Animal and vegetable fats and oils — Determination of saponification value

1 Scope

This document specifies a method for the determination of the saponification value of animal and vegetable fats and oils. The saponification value is a measure of the free and esterified acids present in fats and fatty acids.

The method is applicable to refined and crude vegetable and animal fats.

If mineral acids are present, the results given by this method are not interpretable unless the mineral acids are determined separately.

The saponification value can also be calculated from fatty acid data obtained by gas chromatography analysis as given in Annex B. For this calculation, it is necessary to be sure that the sample does not contain major impurities or is thermally degraded.

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

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
saponification value
I_s
number of milligrams of potassium hydroxide required for the saponification of 1 g of the product tested

4 Principle

The test sample is saponified by boiling under reflux with an excess of ethanolic potassium hydroxide, followed by titration of the excess potassium hydroxide with standard volumetric hydrochloric acid solution.

5 Reagents

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

5.1 Ethanol, volume fraction φ = 95 %.

5.2 Potassium hydroxide, c(KOH) = 0,5 mol/l solution in ethanol.

This solution shall be colourless or straw yellow. A stable colourless solution can be prepared by either of the following procedures.

a) Reflux 1 l of ethanol (5.1) with 8 g of potassium hydroxide and 5 g of aluminium pellets for 1 h, then distil immediately. Dissolve the required amount of potassium hydroxide (approximately 35 g) in the distillate. Allow to stand for several days, then decant the clear supernatant liquid from the precipitated potassium carbonate into a brown-glass stock bottle.

b) Add 4 g of aluminium *tert*-butylate to 1 l 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. Allow to stand for several days, and then decant the clear supernatant liquid from the precipitated potassium carbonate into a brown-glass stock bottle.

5.3 Hydrochloric acid, standard volumetric solution, c(HCl) = 0,5 mol/l.

5.4 Alkali blue 6B solution, ρ = 2,5 g/100 ml in ethanol (5.1).

5.5 Phenolphthalein solution, ρ = 0,1 g/100 ml in ethanol (5.1).

Phenolphthalein is classified as CMR substance and should only be used when no alternative is available.

5.6 Boiling aids.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Conical flask, of 250 ml capacity, made of alkali-resistant glass and having a ground neck.

6.2 Reflux condenser, with a ground glass joint that fits the conical flask (6.1).

6.3 Heating device (e.g. a water-bath, electric hot-plate or other suitable apparatus). A naked flame is not suitable.

6.4 Burette, capacity 50 ml, graduated in 0,1 ml divisions or **automatic burette**.

6.5 Pipette, capacity 25 ml or an **automatic pipette**.

6.6 Analytical balance, readability 0,000 1 g, weighing precision 0,001 g.

7 Sampling

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

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

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

The test samples are carefully mixed and filtered if visible impurities are present. If filtration is necessary, this shall be mentioned in the test report.

9 Procedure

9.1 Test portion

Weigh, to the nearest 5 mg, approximately 2 g of the test sample (see Clause 8) into a conical flask (6.1).

The test portion of 2 g has been determined on the basis of saponification values of 170 to 200. For other saponification values, the mass should be altered accordingly so that approximately half the ethanolic potassium hydroxide solution is neutralized. Recommendations for the mass of the test portion are given in Table 1.

Table 1 — Mass of test portion

Expected saponification value	Mass of test portion
150 to 200	2,2 to 1,8 g
200 to 250	1,7 to 1,4 g
250 to 300	1,3 to 1,2 g
> 300	1,1 to 1,0 g

9.2 Determination

9.2.1 Using a pipette (6.5), add to the test portion 25,0 ml of the ethanolic potassium hydroxide solution (5.2) and some boiling aids (5.6). Connect the reflux condenser (6.2) to the flask, place the flask on the heating device (6.3) and boil gently, shaking from time to time, for 60 min or for 2 h in the case of oils and fats having a high melting point and which are difficult to saponify.

9.2.2 Add to the hot solution 0,5 ml to 1 ml of the colour indicator solution (5.4 or 5.5) and titrate with the standard volumetric hydrochloric acid solution (5.3) until the colour of the indicator changes at the equivalence point. If the solution is strongly coloured, phenolphthalein (5.5) shall not be used as indicator.

9.3 Blank test

Carry out a blank test following the procedure specified in 9.2, using another 25,0 ml of the ethanolic potassium hydroxide solution (5.2) but omitting the test portion.

10 Expression of results

The saponification value, I_s, is given by Formula (1):

$$I_s = \frac{(V_0 - V_1) \times c \times 56,1}{m} \quad (1)$$

where

V_0 is the volume, in millilitres, of the standard volumetric hydrochloric acid solution (5.3) used for the blank test;

V_1 is the volume, in millilitres, of the standard volumetric hydrochloric acid solution (5.3) used for the determination;

c is the exact concentration, in moles per litre, of the standard volumetric hydrochloric acid solution (5.3);

m is the mass, in grams, of the test portion (9.1).

Take as the result the arithmetic mean of the two determinations, provided that the requirement for repeatability (see Clause 11) is satisfied.

Express the result as a whole number. The reporting unit is mg KOH/g fat.

11 Precision

11.1 Results of interlaboratory test

An interlaboratory test carried out at the international level in 2000 by DIN (see Annex A), in which 22 laboratories participated, each of which carried out two determinations on each sample, gave the statistical results (evaluated in accordance with ISO 5725-1 and ISO 5725-2) shown in Table A.1.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method in 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 Table A.1.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method in 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 Table A.1.

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) whether filtration of the test sample(s) was necessary;

d) the test method used, with reference to this document, i.e. ISO 3657:2020;

e) a statement of which indicator has been used, 5.4 or 5.5;

f) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

g) the test result(s) obtained or, if the repeatability has been checked, the final result obtained.

Annex A
(informative)

Results of the interlaboratory test

An international collaborative test involving 22 laboratories in 8 countries was carried out on 5 samples:

— A: coconut oil;

— B: palm oil;

— C: rapeseed oil;

— D: medium chain triglyceride (MCT) oil;

— E: mixture by volume of 60 % A and 40 % D.

The test was organized by DIN in 2000 and the results obtained were subjected to statistical analysis in accordance with ISO 5725-2 to give the precision data shown in Table A.1.

Table A.1 — Summary of statistical results

Parameter	Rapeseed oil	Palm oil	Coconut oil	60 % A + 40 % D	MCT oil
Number of participating laboratories (N)	22	22	22	22	20
Number of laboratories retained after eliminating outliers (n)	19	17	20	18	16
Number of individual test results of all laboratories on each sample (z)	38	34	40	36	32
Mean value ($\bar{I}_S$)	190,2	199,5	256,8	287,5	334,1
Repeatability standard deviation (s_r)	0,7	0,6	0,7	0,7	1,4
Repeatability coefficient of variation ($C_{V,r}$), %	0,4	0,3	0,3	0,2	0,4
Repeatability limit (r)	1,1	0,8	0,8	0,6	1,6
Reproducibility standard deviation (s_R)	1,8	2,0	4,2	2,4	2,9
Reproducibility coefficient of variation ($C_{V,R}$), %	0,9	1,0	1,6	0,8	0,9
Reproducibility limit (R)	2,5	2,8	4,5	2,2	2,5