
**Animal and vegetable fats and oils —
Determination of saponification value**

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





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Published in Switzerland

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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.

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 $\varphi = 95\%$.

5.2 Potassium hydroxide, $c(\text{KOH}) = 0,5 \text{ mol/l}$ 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, $c(\text{HCl}) = 0,5 \text{ mol/l}$.

5.4 Alkali blue 6B solution, $\rho = 2,5 \text{ g/100 ml}$ in ethanol (5.1).

5.5 Phenolphthalein solution, $\rho = 0,1 \text{ g/100 ml}$ 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

Annex B (informative)

Calculation of saponification value from fatty acid composition data

B.1 General

The formulae in [B.3](#) to [B.6](#) provide a mathematical approach to the calculation of the saponification value of fats and oils and their constituent acylglycerols using the fatty acid composition given as fatty acids, fatty acid methyl esters or other fatty acid esters.

The formulae given are thus suitable for use on a computer. A worked example is given where a manual calculation is to be carried out (see [B.7](#)).

Fatty acid methyl esters shall be determined using ISO 12966-2, ISO 12966-3 and ISO 12966-4.

B.2 Symbols

$C_{F(i)}$	carbon number of <i>i</i> th fatty acid or ester
$C_{T(i)}$	carbon number of <i>i</i> th triacylglycerol
<i>i</i>	particular fatty acid or ester or triacylglycerol
I_{sc}	calculated saponification value
<i>k</i>	integer constant for fatty acid derivative
\overline{M}	mean relative molecular mass of all fatty acids in the test sample
M_{CH_2}	relative molecular mass of CH ₂ (14,026 7)
M_{H_2}	relative molecular mass of H ₂ (2,015 9)
M_{HCOOH}	relative molecular mass of HCOOH (46,025 5)
$M_{F(i)}$	relative molecular mass of <i>i</i> th fatty acid or ester
$M_{T(i)}$	relative molecular mass of <i>i</i> th triacylglycerol
$n_{F(i)}$	number of double bonds in <i>i</i> th fatty acid or ester
$n_{T(i)}$	number of double bonds in <i>i</i> th triacylglycerol
$w_{F(i)}$	percentage mass fraction of <i>i</i> th fatty acid or ester
$w_{T(i)}$	percentage mass fraction of <i>i</i> th triacylglycerol
w_U	mass fraction of unsaponifiable matter
$x_{F(i)}$	percentage mole fraction of <i>i</i> th fatty acid or ester
$x_{T(i)}$	percentage mole fraction of <i>i</i> th triacylglycerol

B.3 Calculation of relative molecular mass of fatty acids or esters

$$M_{F(i)} = M_{CH_2} \cdot (C_{F(i)} - 1) - (M_{H_2} \cdot n_i) + M_{HCOOH} + (M_{CH_2} \cdot k)$$

which equates to:

$$M_{F(i)} = 14,0267 \cdot (C_{F(i)} - 1) - (2,0159 \cdot n_i) + 46,0255 + (14,0267 \cdot k)$$

where

$k = 0$ for acid;

$k = 1$ for methyl ester;

$k = 2$ for ethyl ester;

$k = 3$ for propyl ester;

$k = 4$ for butyl ester.

Or for acids for which the relative molecular mass is given by the user:

$$M_{F(i)} = M_{F(i)} + (14,0267 \cdot k)$$

B.4 Calculation of the relative molecular mass of triacylglycerols

$$M_{T(i)} = M_{F1(i)} + M_{F2(i)} + M_{F3(i)} + (M_{C_3H_5} + 3 \cdot M_{CO_2} + 3 \cdot M_H) - (3 \cdot M_{HCOOH} + M_{CH_2} \cdot k)$$

which equates to:

$$M_{T(i)} = M_{F1(i)} + M_{F2(i)} + M_{F3(i)} + 176,1248 - 3 \cdot (46,0255 + 14,0267 \cdot k)$$

$$M_{T(i)} = 14,0267 \cdot [C_{T(i)} - 3] - 2,0159 \cdot n_{T(i)} + 176,1248$$

B.5 Calculation of the mean relative molecular mass of triacylglycerols

$$\bar{M} = \sum_i x_{F(i)} \cdot M_{F(i)}$$

$$\bar{M} = \sum_i x_{T(i)} \cdot M_{T(i)}$$

B.6 Calculation of the saponification value

From the definition of saponification value (3.1), for triacylglycerols:

$$I_{sc} = \frac{3 \cdot 56,1 \cdot 1000}{\bar{M}}$$

For diacylglycerols, the factor 3 is replaced by 2, and for fatty acids and monoacylglycerols by 1.

B.7 Example

B.7.1 Fatty acid methyl ester composition of a sample

A sample with the following methyl ester composition is used: 16:0 (10,6 % mass fraction), 18:0 (4,2 % mass fraction), 18:1 (22,7 % mass fraction), 18:2 (54,5 % mass fraction), 18:3 (8,0 % mass fraction).

B.7.2 Calculation of the relative molecular mass of the methyl esters ($k = 1$)

$$M_{F(i)} = 14,0267 \cdot (C_{F(i)} - 1) - (2,0159 \cdot n_i) + 46,0255 + (14,0267 \cdot 1)$$

Some relative molecular masses are listed in the following:

palmitic acid	270,45
stearic acid	298,51
oleic acid	296,49
linoleic acid	294,47
linolenic acid	292,46

B.7.3 Calculation of the relative molecular mass of triacylglycerols for $k = 1$

The relative molecular mass of a triacylglycerol (TAG) is the sum of the three fatty acid methyl ester masses minus the mass of four hydrogen atoms (4,031 8).

$$M_{T(i)} = m_{F1(i)} + m_{F2(i)} + m_{F3(i)} - 4,0318$$

For 16:0 TAG, this gives $270,45 + 270,45 + 270,45 - 4,0318 = 807,32$.

For 18:0 TAG, this gives $298,51 + 298,51 + 298,51 - 4,0318 = 891,50$.

For 18:1 TAG, this gives $296,49 + 296,49 + 296,49 - 4,0318 = 885,44$.

For 18:2 TAG, this gives $294,47 + 294,47 + 294,47 - 4,0318 = 879,38$.

For 18:3 TAG, this gives $292,46 + 292,46 + 292,46 - 4,0318 = 873,35$.

B.7.4 Calculation of the mean relative molecular mass

For the manual calculation, to save effort, a slight approximation is made by assuming that $w_{T(i)} = w_{F(i)}$, i.e. that the mass fractions of the equivalent TAGs are the same as the mass fractions of the fatty acid methyl esters.

Mean relative molecular mass for all triacylglycerols is:

$$\overline{M}_T = \frac{(10,6 \cdot 806,72 + 4,2 \cdot 891,50 + 22,7 \cdot 885,44 + 54,5 \cdot 879,38 + 8,0 \cdot 873,35)}{100}$$

$$\overline{M}_T = 872,42$$

B.7.5 Calculation of the saponification value

$$I_{sc} = \frac{3 \cdot 1000 \cdot 56,1}{872,42} = 193$$

NOTE The result is expressed as a whole number.

B.8 Corrections for free fatty acids, partial glycerols, and unsaponifiable matter

For free fatty acids (FFA) and partial glycerols (PG), the appropriate molecular mass and saponification values are calculated from the formulae in [B.3](#) to [B.6](#). Then the overall molecular mass and saponification value are calculated by mass balance from the percentages of FFA and PG given.

A correction for unsaponifiable matter may be made by the formula:

$$I_s = I_{sc} \cdot \frac{(100 - w_u)}{100}$$

Bibliography

- [1] ISO 5555, *Animal and vegetable fats and oils — Sampling*
- [2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [4] ISO 12966-2, *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 2: Preparation of methyl esters of fatty acids*
- [5] ISO 12966-3, *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)*
- [6] ISO 12966-4, *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography*

