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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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Foreword

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This fourth edition cancels and replaces the third edition (ISO 3657:2002), which has been technically revised.

Animal and vegetable fats and oils — Determination of saponification value

1 Scope

This International Standard 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 liquid 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, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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.

3.1
saponification value
number of milligrams of potassium hydroxide required for the saponification of 1 g of the product tested

Note 1 to entry: The saponification value is a dimensionless quantity. The usual abbreviation is SV.

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

5.4 Phenolphthalein solution, $\rho = 0{,}1$ g/100 ml in ethanol (5.1).

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

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 which 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 International Standard. A recommended sampling method is given in ISO 5555.

It is important that the laboratory receive a truly representative sample which 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 (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 phenolphthalein solution (5.4) and titrate with the standard volumetric hydrochloric acid solution (5.3) until the pink colour of the indicator just disappears. If the solution is strongly coloured, use 0,5 ml to 1 ml of alkali blue 6 B solution (5.4).

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 the equation

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

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 (Clause 11) is satisfied.

Express the result as a whole number.

11 Precision

11.1 Results of interlaboratory test

An interlaboratory test, carried out at the international level in 2000 by DIN, 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[2] and ISO 5725-2[3]) 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 test sample(s) was necessary;

d) the test method used, with reference to this International Standard (ISO 3657:2013);

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

f) all operating details not specified in this International Standard, 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 an interlaboratory test

An international collaborative test involving 22 laboratories in eight countries was carried out on five 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[3] 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 equations 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 equations given are thus very suitable for use on a computer. A worked example is given where a manual calculation is to be carried out (B.7).

Fatty acid methyl esters shall be determined using ISO 5508,[4] ISO 12966-2,[5] and ISO 12966-3.[6]

B.2 Symbols and abbreviated terms

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

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

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

which equates to:

$$M_{\mathrm{F}(i)} = 14{,}026\,7 \cdot \left(C_{\mathrm{F}(i)} - 1\right) - \left(2{,}015\,9 \cdot n_i\right) + 46{,}025\,5 + \left(14{,}026\,7 \cdot k\right)$$

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 whose relative molecular mass is given by the user

$$M_{\mathrm{F}(i)} = M_{\mathrm{F}(i)} + \left(14{,}026\,7 \cdot k\right)$$

B.4 Calculation of the relative molecular mass of triacylglycerols

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

which equates to:

$$M_{\mathrm{T}(i)} = M_{\mathrm{F1}(i)} + M_{\mathrm{F2}(i)} + M_{\mathrm{F3}(i)} + 176{,}124\,8 - 3 \cdot (46{,}025\,5 + 14{,}026\,7 \cdot k)$$

$$M_{\mathrm{T}(i)} = 14{,}026\,7 \cdot \left[C_{\mathrm{T}(i)} - 3\right] - 2{,}015\,9 \cdot n_{\mathrm{T}(i)} + 176{,}124\,8$$

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

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

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

B.6 Calculation of the saponification value

From the definition of saponification value (3), for triacylglycerols:

$$I_{\mathrm{sc}} = \frac{3 \cdot 56{,}1 \cdot 1\,000}{\overline{M}}$$

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 ($k = 1$)

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

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).

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

For 16:0 TAG, this gives 270,45 + 270,45 + 270,45 − 4,0318 = 806,72

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, we make a slight approximation by assuming that $w_{\mathrm{T(i)}} = w_{\mathrm{F}(i)}$, 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

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

$$\overline{M}_{\mathrm{T}} = 872{,}42$$

B.7.5 Calculation of the saponification value

$$I_{\mathrm{sc}} = \frac{3 \cdot 1\,000 \cdot 56{,}1}{872{,}42} = 193$$