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Animal and vegetable fats and oils — Determination of oxidative stability (accelerated oxidation test)

Corps gras d’origines animale et végétale — Détermination de la stabilité à l’oxydation (essai d’oxydation accéléré)

Reference number
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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 third edition cancels and replaces the second edition (ISO 6886:2006), of which it constitutes a minor revision.

Animal and vegetable fats and oils — Determination of oxidative stability (accelerated oxidation test)

1 Scope

This International Standard specifies a method for the determination of the oxidative stability of fats and oils under extreme conditions that induce rapid oxidation: high temperature and high air flow. It does not allow determination of the stability of fats and oils at ambient temperatures, but it does allow a comparison of the efficacy of antioxidants added to fats and oils.

The method is applicable to both virgin and refined animal and vegetable fats and oils. Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this International Standard.

NOTE The presence of volatile fatty acids and volatile acidic oxidation products prevents accurate measurement.

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
induction period
time between the start of the measurement and the time when the formation of oxidation products rapidly begins to increase

3.2
oxidative stability
induction period, expressed in hours, determined according to the procedure specified in this International Standard

Note 1 to entry: A temperature of 100 °C to 120 °C is usually applied for the determination of oxidative stability. Depending on the oxidative stability of the sample under test, or when an extrapolation of regression is required, the determination may be carried out at other temperatures. The optimal induction period is between 6 h to 24 h. A temperature increase or decrease of 10 °C decreases or increases the induction period by a factor of approximately 2.

3.3
conductivity
ability of a material to conduct electric current

4 Principle

A stream of purified air is passed through the sample, which has been brought to a specified temperature. The gases released during the oxidation process, together with the air, are passed into a flask containing water that has been demineralized or distilled and contains an electrode for measuring the conductivity. The electrode is connected to a measuring and recording device. The end of the induction period is indicated when the conductivity begins to increase rapidly. This accelerated increase is caused by the accumulation of volatile fatty acids produced during oxidation.

5 Reagents and materials

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

5.1 Molecular sieve, beads of approximately 1 mm diameter, pore size 0,3 nm, with moisture indicator.

The molecular sieve should be dried in an oven set at 150 °C and then cooled down to room temperature in a desiccator.

5.2 Acetone.

5.3 Alkaline cleaning solution, for laboratory glassware.

5.4 Glycerol.

5.5 Thermostable oil.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Appliance for the determination of oxidative stability

See Figures 1 and 2 for diagrammatic representations.

NOTE An appliance for determining oxidative stability can be obtained commercially under the trade name Rancimat, from Metrohm AG, Herisau, Switzerland, or the OSI equipment from Omnion Inc., USA.[1)]

6.1.1 Air filter, comprising a tube fitted with filter paper at each end and filled with molecular sieve (5.1), connected to the suction end of a pump.

6.1.2 Gas diaphragm pump, with an adjustable flow rate of 10 l/h, in combination with an apparatus to control the flow rate, manually or automatically, with a maximum deviation of ±1,0 l/h from the set value.

NOTE For the OSI instrument, a pressure of 5,5 psi is equivalent to a flow of approximately 10 l/h.

6.1.3 Aeration vessels of borosilicate glass (usually eight), connected to a sealing cap.

The sealing cap shall be fitted with a gas inlet and outlet tube. The cylindrical part of the vessel shall preferably be narrower by a few centimetres below the top in order to break up any emerging foam. An artificial foam blocker (e.g. glass ring) may also be used for this purpose.

6.1.4 Closed measurement cells (usually eight), of approximately 150 ml capacity, with a gas inlet tube extending to the bottom inside of the vessel.

The cell shall be provided at the top with ventilation holes.

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1) Rancimat (www.metrohm.com) and OSI (Omnion) (http://world.std.com/~omnion/) are examples of suitable equipment available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this equipment.

6.1.5 Electrodes (usually eight), for measuring conductivity with a measuring range of 0 µS/cm to 300 µS/cm, aligned with the dimensions of the measurement cell (6.1.4).

6.1.6 Measuring and recording apparatus, comprising an amplifier and a recorder for registering the measuring signal of each of the electrodes (6.1.5).

NOTE A computer-controlled central processing unit is used with Rancimat and OSI (Omnion).

6.1.7 Certified and calibrated contact thermometer, graduated in 0,1 °C, or **Pt 100 element (platinum resistance thermometer)** to measure the block temperature, with attachments for a control relay connection and an adjustable heating element; temperature range 0 °C to 150 °C.

6.1.8 Heating block, made of cast aluminium, adjustable to a temperature of up to 150 °C ± 0,1 °C.

The block shall be provided with holes (usually eight) for the aeration vessels (6.1.3), and an aperture for the contact thermometer (6.1.7).

Alternatively, a **heating bath** may be used, filled with oil, suitable for temperatures up to 150 °C and adjustable to the nearest 0,1 °C.

6.2 Certified and calibrated thermometer or **Pt100** element, with a temperature range up to 150 °C, graduated in 0,1 °C.

6.3 Measuring pipettes, of capacity 50 ml and 5 ml.

6.4 Oven, capable of being maintained at a temperature of up to 150 °C ± 3 °C.

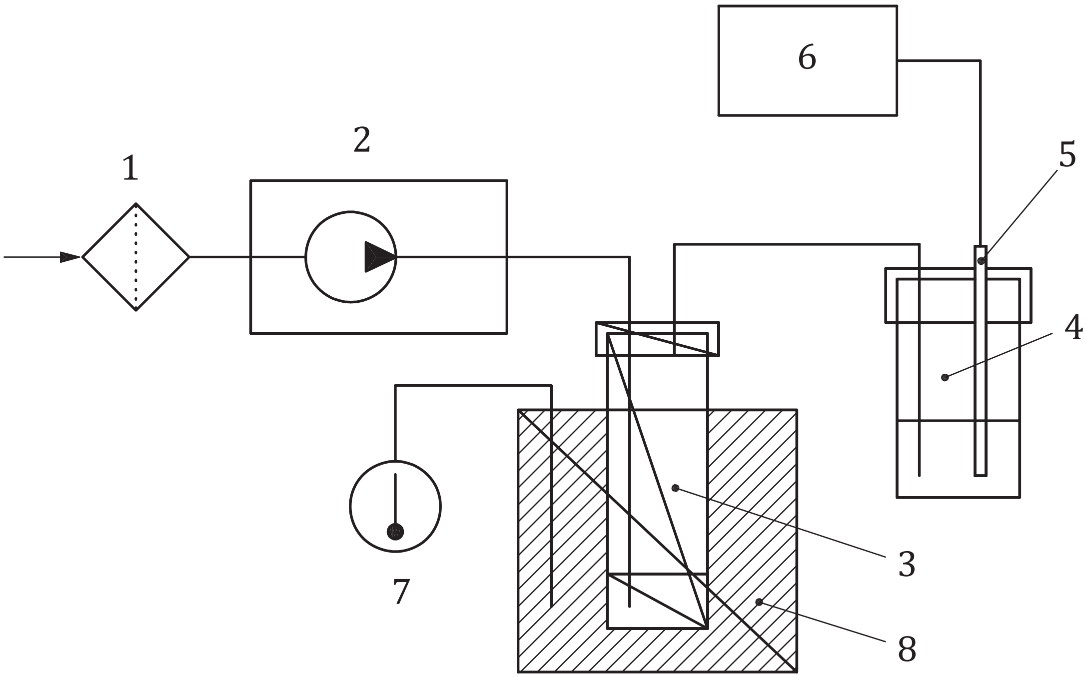
6.5 Connecting hoses, flexible and made of inert material {polytetrafluoroethylene [Polytetrafluoroethylene (PTFE)] or silicone}.

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.[1]

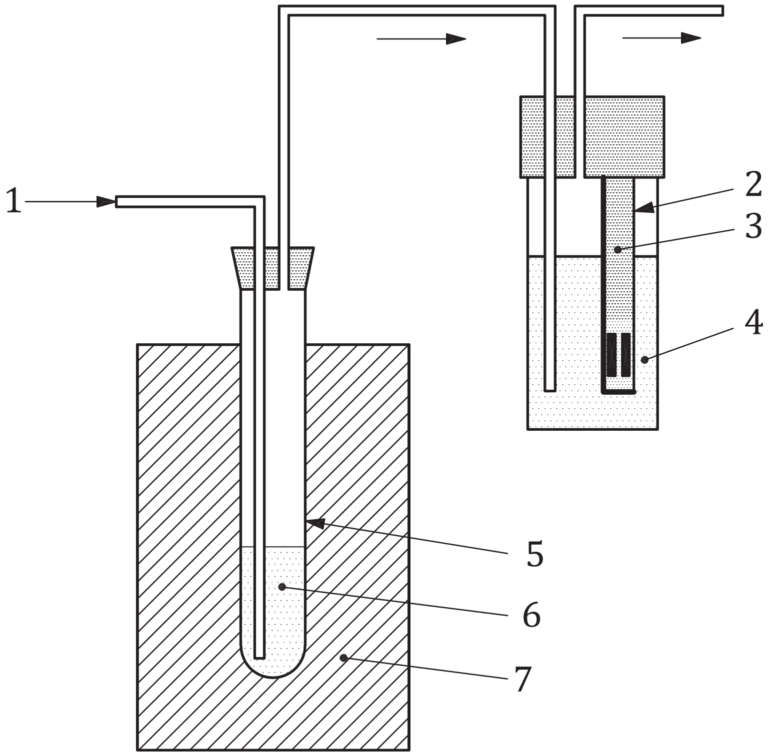
Store the sample in the dark at about 4 °C.



Key

1	air filter (6.1.1)
2	gas diaphragm pump with flow rate control (6.1.2)
3	aeration vessel (6.1.3)
4	measurement cell (6.1.4)
5	electrode (6.1.5)
6	measuring and recording apparatus (6.1.6)
7	thyristor and contact thermometer (6.1.7)
8	heating block (6.1.8)

Figure 1 — Diagrammatic representation of the apparatus



Key

1	air	5	aeration vessel
2	measurement cell	6	sample
3	electrode	7	heating block
4	measuring solution		

Figure 2 — Diagrammatic representation of heating block, reaction vessel and measurement cell

8 Preparation of test sample and apparatus

8.1 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

Remove the required quantity from the centre of the carefully homogenized sample using a pipette.

Heat semisolid and solid samples to a temperature somewhat above their melting points and mix carefully. Overheating should be avoided. The pipette shall be at the same temperature as the sample.

8.2 Preparation of apparatus

8.2.1 Cleaning procedure

Wash the aeration vessels, measurement cells and their inlet and outlet tubes at least three times with acetone in order to remove as much of the organic residue as possible. Rinse with tap water.

The cleanliness of the aeration vessels is paramount in achieving correct induction periods. All traces of oxidized oils from previous runs shall be removed.

Fill the vessels completely with an aqueous alkaline laboratory glass-cleaning solution and mount the inlet tubes. Store the vessels for at least 2 h at 70 °C.

Rinse the purified vessels and their inlet and outlet tubes thoroughly with tap water and finally with demineralized or distilled water. Dry them in an oven (6.4) for at least 1 h at 110 °C.

NOTE If disposable aeration vessels are used, the cleaning procedure described above is not necessary.

8.2.2 Determination of temperature correction

The difference between the actual temperature of the sample and the temperature of the heating block is called the temperature correction, ΔT. For the determination of ΔT, an external calibrated temperature sensor shall be used.

For the Rancimat, the temperature calibration equipment may be purchased from Metrohm. But, in all cases, a precision thermometer shall be used for the temperature correction.

The correct temperature in the aeration vessels is of great importance for achieving repeatable and reproducible results. Due to the cold air that bubbles through the sample, it is necessary to set a slightly higher temperature for the heating block. Generally, the temperature setting shall be 1 °C to 2 °C higher for the heating block to achieve the desired temperature of 100 °C, 110 °C or 120 °C in the oil.

Before starting the determination of ΔT, the heating-block shall be switched on and the target temperature shall have been reached.

Fill one reaction vessel with 5 g of thermostable oil. Insert the temperature sensor through the cap into the reaction vessel. Use distance clips to keep the sensor away from the air inlet.

CAUTION — The sensor shall be immersed completely in the oil sample and shall not touch the bottom of the vessel.

Insert the complete vessel into the heating-block and connect the air supply.

If the value of the measured temperature is constant, calculate the temperature correction, ΔT:

$$\Delta T = T_{\text{block}} - T_{\text{sensor}} \tag{1}$$

where

T_{block} is the temperature of the heating block;

T_{sensor} is the measured temperature in the reaction vessel.

The corrected temperature of the block, T'_{block}, is then given by

$$T'_{\text{block}} = T_{\text{target}} + \Delta T \tag{2}$$

where T_{target} is the target temperature.

After this temperature correction has been applied, the temperature in the reaction vessel should be equal to the target temperature.

9 Procedure

9.1 Set up the apparatus as shown in Figure 1. If the apparatus is available commercially, follow the manufacturer's instructions.

9.2 Attach the gas diaphragm pump (6.1.2) and adjust the flow to exactly 10 l/h. Then switch the pump off again. Commercially available apparatus may be used to control the set flow automatically.

NOTE For the OSI instrument, a pressure of 5,5 psi is equivalent to a flow of approx. 10 l/h.

9.3 Bring the heating block (6.1.8) up to the desired temperature (usually 100 °C, but see 8.2.2) using the thyristor and contact thermometer (6.1.7) or by using an electronic controller. The temperature shall be maintained within a range of ±0,1 °C during the test period.

Pour some glycerol (5.4) into the holes of the heating block (6.1.8) in order to promote heat transfer if necessary.

If a heating bath (6.1.8) is used, bring it to the desired temperature and check as described in 8.2.2.

9.4 Fill the measurement cells (6.1.4) with 50 ml of distilled or demineralized water using a measuring pipette (6.3).

NOTE At temperatures above 20 °C, volatile carboxylic acids can evaporate from the water in the measurement cell. This can lead to a decrease in the conductivity of the aqueous solution. The rapidly rising part of the conductivity curve will, therefore, produce a deviant shape, so it becomes impossible to determine the tangent on this part of the curve (see Reference [2]).

9.5 Check the electrodes (6.1.5) and adjust their signals, using a calibration potentiometer, so that they are on the zero axis of the recorder paper.

Set the paper rate at 10 mm/h and the measuring frequency at one measuring point per 20 s. Set the measuring value of 200 µS/cm at the maximum result of 100 %.

If it is not possible to adjust the paper rate to 10 mm/h, 20 mm/h should be used and this should be reported on the recorder paper.

NOTE Commercially available apparatus might acquire the data via a computer.

9.6 Using a pipette (6.3), weigh to the nearest 0,01 g, 3,0 g of the conditioned sample (see 8.1) into an aeration vessel (6.1.3).

9.7 Switch on the gas diaphragm pump (6.1.2) and set the flow again at exactly 10 l/h (or 5,5 psi). Connect the air inlet tube and outlet tube to the aeration vessels and the measurement cells, using the connecting hoses (6.5).

NOTE For the OSI instrument, a pressure of 5,5 psi is equivalent to a flow of approximately 10 l/h.

9.8 Place the aeration vessel with the sealing cap (6.1.3) into the hole intended for it in the heating block or into the heating bath (6.1.8), both of which shall have reached the required temperature.

The preparation steps 9.7 and 9.8 should be carried out as quickly as possible. Then, immediately start the automatic data recorder or note on the recorder paper the time that measurements were started.

9.9 Finish the measurements when the signal has reached 100 % of the recorder scale, usually 200 µS/cm.

9.10 During the determination, carry out the following precautions:

a) check the setting of the flow meter and adjust where necessary in order to ensure a constant flow;

b) check the colour of the molecular sieve (5.1) of the air filter.

Repeat measurements shall be carried out if the molecular sieve changes colour during the test. It is recommended to change the molecular sieve prior to each run.

10 Calculations

10.1 Manual calculation

Draw the optimum tangent along the first, moderately increasing part of the curve. Draw the optimum tangent along the upper part of the rapidly increasing part of the curve. (For more details, see Figure A.1.) Carry out the determination again if it is not possible to draw the optimum tangent.

Determine the oxidative stability by reading off the time at the point where the two lines intersect (the induction period).

10.2 Automatic calculation

Commercial equipment allows automatic calculation of the induction period by using the maximum of second derivative of the curve. (For more details, see Annex A.)

Express the oxidative stability in hours, to the nearest 0,1 h.

NOTE Examples of conductivity curves are shown in Figure A.1. A curve which rises very rapidly can be the result of the temperature of the solution in the measurement cell being too high, causing volatile carboxylic acid to evaporate from the solution (see Reference [2]).

11 Precision

11.1 Results of interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex B. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 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, will in not more than 5 % of cases be greater than 6 % of the arithmetic mean of the two results, for an oxidative stability of between 2 h and 45 h.

11.3 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, will in not more than 5 % of cases be greater than 29 % of the arithmetic mean of the two results, for an oxidative stability of between 2 h and 45 h.

12 Test report

The test report shall specify the following:

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

b) the sampling method used, if known, with reference to this International Standard;

c) the test method used with reference to this International Standard, i.e. ISO 6886;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the result;

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

Annex A
(informative)

Summary of the method and examples of conductivity curves and the determination of induction time

Over the years, a number of methods have been developed for the determination of the oxidative stability of oils and fats. These methods are based on the rate of oxygen absorption by oils and fats (in a liquid state) which have been placed in contact with air.

Oxygen absorption may be measured directly using the Warburg apparatus or indirectly by determination of peroxides or the products dissociated from them during oxidation.

Of the indirect methods of determination, the active oxygen method (AOM) is the oldest. This is based on determination of the peroxide value in line with the progress of the aeration of the sample at 98,7 °C. It specifies the time which elapses until a peroxide value of 100 mmol (active oxygen per 2 kg) has been reached. The swift stability test has been derived from this method. These determinations are very time-consuming and cannot be automated.

In the method described in this International Standard, the oxidation process is in two phases:

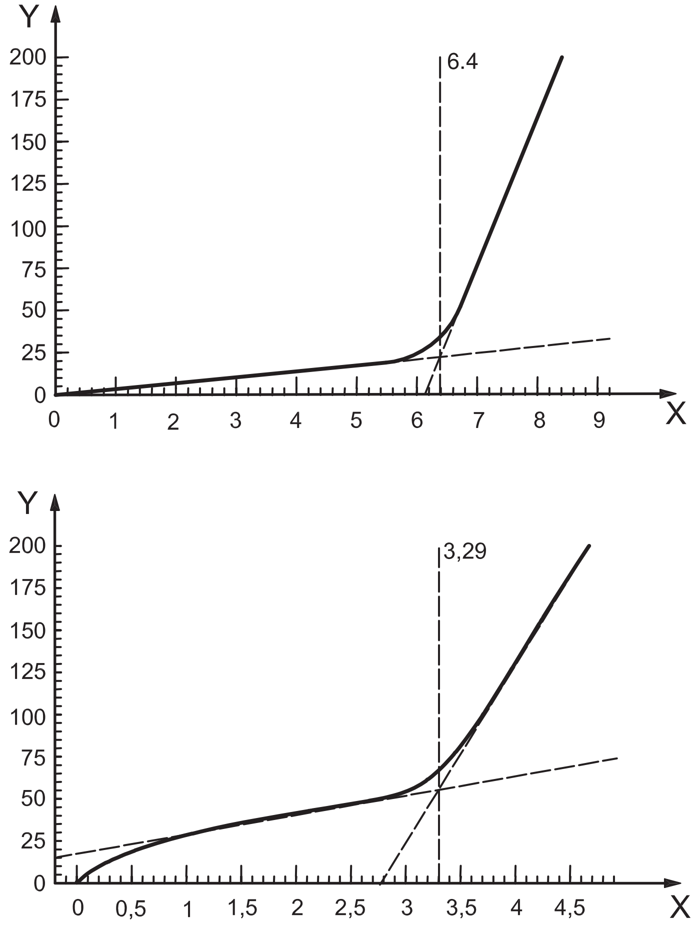
a) the first phase (the induction period) is characterized by slow absorption of oxygen during which peroxides are formed;

b) the second phase (tainted odour and flavour phase) is characterized by rapid absorption in which peroxides are not only formed but these peroxides are then dissociated under the influence of the high temperature. During this phase, products such as aldehydes, ketones and low fatty acids are formed. These products give rise to deviant odour and flavour.

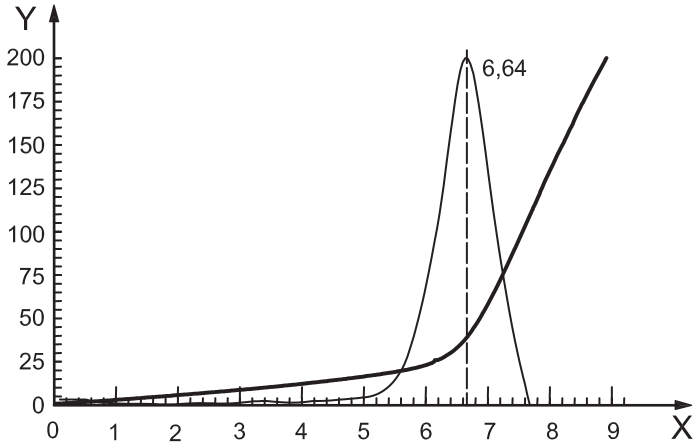
The method described in this International Standard is a conductometric determination of volatile acid dissociation products (mainly formic acid and acetic acid) produced during oxidation.

The procedure was published in 1974 (see Reference [4]). An automated potentiometric determination method was published in 1972 (see Reference [5]).

From the conductivity curve, an induction time is determined in line with the induction time obtained using the AOM, provided that the determinations are carried out at the same temperature. The curves can have very different shapes. Examples of curves are shown in Figure A.1 (taken from Reference [6]).



a) Manual calculations



b) Automatic calculation

Key

X time, h

Y conductivity, µS/cm

Figure A.1 — Examples of conductivity curves

Annex B
(informative)

Results of an interlaboratory test

An international collaborative test involving 21 laboratories in six countries was carried out on four samples at three different temperatures. Three participants used an OSI apparatus and 18 participants used a Rancimat (3 used the Rancimat type 617, 9 used the Rancimat type 679 and 6 used the Rancimat type 743). The test was organized by the Deutsches Institut für Normung (DIN) in 2004. The results obtained were subjected to statistical analysis in accordance with ISO 5725-2[3] to give the precision data shown in Tables B.1 to B.3.

Table B.1 — Summary of statistical results at 120 °C

Samples at 120 °C	**Soybean oil**	**Rape-seed oil**	**Extra virgin olive oil**
Number of participating laboratories	21	21	21
Number of laboratories retained after eliminating outliers	18	20	18
Number of individual test results of all laboratories	36	40	36
Mean value, h	4,17	4,10	20,11
Repeatability standard deviation (s_r)	0,09	0,14	0,25
Repeatability coefficient of variation, %	2,2	3,3	1,2
Repeatability limit (r)	0,25	0,38	0,70
Reproducibility standard deviation (s_R)	0,41	0,48	2,21
Reproducibility coefficient of variation, %	9,9	11,8	11,0
Reproducibility limit (R)	1,16	1,35	6,20

Table B.2 — Summary of statistical results at 110 °C

Samples at 110 °C	**Soybean oil**	**Rape-seed oil**	**Extra virgin olive oil**	**Linseed oil**
Number of participating laboratories	21	21	21	21
Number of laboratories retained after eliminating outliers	18	19	16	18
Number of individual test results of all laboratories	36	38	32	36
Mean value, h	8,01	8,13	45,22	2,82
Repeatability standard deviation (s_r)	0,13	0,16	0,28	0,08
Repeatability coefficient of variation, %	1,6	1,9	0,6	2,8
Repeatability limit (r)	0,36	0,44	0,78	0,22
Reproducibility standard deviation (s_R)	0,75	0,76	3,74	0,29
Reproducibility coefficient of variation, %	9,3	9,3	8,3	10,3
Reproducibility limit (R)	2,09	2,12	10,47	0,81

Table B.3 — Summary of statistical results at 100 °C

Sample E at 100 °C	**Linseed oil**
Number of participating laboratories	19
Number of laboratories retained after eliminating outliers	18
Number of individual test results of all laboratories	36
Mean value, h	5,55
Repeatability standard deviation (s_r)	0,09
Repeatability coefficient of variation, %	1,6
Repeatability limit (r)	0,25
Reproducibility standard deviation (s_R)	0,44
Reproducibility coefficient of variation, %	8,0
Reproducibility limit (R)	1,24

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