
**Animal and vegetable fats and oils —
Determination of peroxide value —
Potentiometric end-point determination**

*Corps gras d'origines animale et végétale — Détermination de l'indice
de peroxyde — Détermination avec point d'arrêt potentiométrique*



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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 27107 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Introduction

Over many years, various methods have been developed for the determination of peroxides in fats and oils. Their general principle is the liberation of iodine from potassium iodide in an acid medium. The method according to Wheeler (Reference [6]) was first adopted in standards more than 50 years ago by different bodies, and is widely used to control commodities by producers, receivers, and official laboratories. In national and international food legislation (including Codex Alimentarius), acceptable limits for peroxide values are often specified. Due to anomalies in the reproducibility of the results, it was noticed that there are slight differences between the standardized methods. A very important point is the dependence of the result on the amount of sample used for the determination. As the determination of the peroxide value (PV) is a highly empirical procedure, ISO/TC 34/SC 11 has decided to fix the sample mass at 5 g for $PV > 1$, and at 10 g for $PV \leq 1$, and to limit the applicability of this method to animal and vegetable fats and oils with peroxide values from 0 mmol to 15 mmol of active oxygen per kilogram. The users of this International Standard should be aware that the results obtained can be slightly lower than with previous standards.

Animal and vegetable fats and oils — Determination of peroxide value — Potentiometric end-point determination

1 Scope

This International Standard specifies a method for the potentiometric end-point determination of the peroxide value, in milliequivalents of active oxygen per kilogram, of animal and vegetable fats and oils.

The method is applicable to all animal and vegetable fats and oils, fatty acids and their mixtures with peroxide values from 0 meq to 30 meq of active oxygen per kilogram. It is also applicable to margarines and fat spreads with varying water content. The method is not applicable to milk fats or lecithins.

NOTE A method for the iodometric (visual) determination of the peroxide value is given in ISO 3960. For milk fats, a method is specified in ISO 3976.

2 Normative references

The following referenced documents are indispensable for the application of this document. 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

peroxide value

PV

quantity of those substances in the sample, expressed in terms of active oxygen, that oxidize potassium iodide under the conditions specified in this International Standard

NOTE The peroxide value is usually expressed in milliequivalents of active oxygen per kilogram of oil, but it may also be expressed (in SI units) as millimoles of active oxygen per kilogram of oil. The value expressed in millimoles of active oxygen per kilogram is half that expressed in milliequivalents of active oxygen per kilogram. Multiplication of the peroxide value (milliequivalents of active oxygen per kilogram) by the equivalent mass of oxygen (equalling 8) gives the active oxygen mass fraction in milligrams per kilogram of oil.

4 Principle

The sample is dissolved in isooctane and glacial acetic acid, and potassium iodide is added. The iodide liberated by the peroxides is determined volumetrically with a sodium thiosulfate standard solution. The end-point of the titration is determined electrochemically.

5 Reagents

WARNING — Attention is drawn to national regulations that specify the handling of hazardous substances, and users' obligations thereunder. Technical, organizational and personal safety measures shall be followed.

Unless otherwise specified, use only reagents of recognized analytical grade. All reagents shall be free of dissolved oxygen.

5.1 Water, distilled, boiled, and cooled to 20 °C.

5.2 Glacial acetic acid, mass fraction 100 %, degassed in an ultrasonic bath under vacuum or by purging with a stream of pure and dry inert gas (carbon dioxide or nitrogen).

5.3 Isooctane (2,2,4-trimethylpentane), degassed in an ultrasonic bath under vacuum or by purging with a stream of pure and dry inert gas (carbon dioxide or nitrogen).

5.4 Glacial acetic acid/isooctane solution, prepared by mixing 60 ml glacial acetic acid (5.2) and 40 ml isooctane (5.3). Volume fraction of glacial acetic acid: $\varphi = 60 \text{ ml}/100 \text{ ml}$; volume fraction of isooctane: $\varphi = 40 \text{ ml}/100 \text{ ml}$.

The mixture is degassed in an ultrasonic bath under vacuum or by purging with a stream of pure and dry inert gas (carbon dioxide or nitrogen).

5.5 Potassium iodide, free from iodine and iodates.

5.6 Saturated potassium iodide solution, mass concentration $\rho(\text{KI}) = 175 \text{ g}/100 \text{ ml}$.

Dissolve approximately 14 g potassium iodide in approximately 8 g freshly boiled water (5.1) at room temperature. Make sure the solution remains saturated (i.e. some undissolved crystals remain in the container). Store in the dark and prepare freshly every day. Test the solution as follows: add two drops of starch solution to 0,5 ml of the potassium iodide solution in 30 ml of the glacial acetic acid/isooctane solution (5.4). If more than one drop of sodium thiosulfate standard solution (5.7) is needed to form a blue colour, discard the potassium iodide solution.

5.7 0,1 N sodium thiosulfate standard solution, amount of substance concentration $c(\text{Na}_2\text{S}_2\text{O}_3) = 0,1 \text{ mol/l}$.

Use only freshly boiled water (5.1) for the preparation of this solution, possibly purged with nitrogen. This solution can be used for 1 month and shall be stored in an amber-stained bottle.

5.8 0,01 N sodium thiosulfate standard solution, amount of substance concentration $c(\text{Na}_2\text{S}_2\text{O}_3) = 0,01 \text{ mol/l}$.

Pipette (6.3) 100 ml of the 0,1 N sodium thiosulfate standard solution (5.7) into a volumetric flask of capacity 1 000 ml (6.9). Make up to the mark with water (5.1). After homogenization, transfer the obtained 0,01 N sodium thiosulfate standard solution to an amber-stained bottle.

Prepare the 0,01 N sodium thiosulfate standard solution freshly from the 0,1 N sodium thiosulfate standard solution just before use or determine the titre daily. As experience shows, the stability is limited and depends upon the pH value and the content of free carbon dioxide. Use only freshly boiled water (5.1) for the dilution, possibly purged with nitrogen.

5.9 Potassium iodate(V) volumetric standard, secondary reference material, traceable to the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA.

5.10 Hydrochloric acid, amount of substance concentration $c(\text{HCl}) = 4 \text{ mol/l}$.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Automatic titrator with processor, dosing device, stirrer and electrodes.

If other apparatus is used, the procedure shall be optimized for the relevant apparatus. The apparatus shall be able to perform a dynamic titration (fast at the beginning, slow near the end-point). This is necessary to minimize the titration time whilst achieving a slow titration near the end-point.

6.2 Combined platinum electrode.

6.3 Pipettes, of capacities 0,5 ml, 1 ml, 10 ml and 100 ml. Suitable automatic pipettes may also be used.

6.4 Measuring cylinders, of capacities 50 ml and 100 ml.

6.5 Analytical balance, readable to 0,001 g.

6.6 Magnetic stirrer, with magnetic stirring rod of length 25 mm, and heating plate.

6.7 Erlenmeyer flask, of capacity 250 ml.

6.8 Beaker, of capacity 250 ml, and of tall form.

6.9 Volumetric flask, of capacity 1 000 ml.

6.10 Volumetric flask, of capacity 250 ml.

6.11 Volumetric flask, of capacity 500 ml.

6.12 Microwave oven.

6.13 Amber-stained bottles, of capacity 1 000 ml.

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.

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

Homogenize the test sample, preferably without heating and without aeration. Avoid direct solar radiation. Heat solid test samples carefully to 10 °C above their melting point, using a microwave oven. Test samples with visible impurities shall be filtered; the filtration shall be noted in the test report.

Take the test portion for the determination of peroxide value first, before taking test portions for any other test, and determine the peroxide value immediately.

9 Procedure

9.1 General

Carry out all steps in diffuse daylight or in artificial light. Avoid direct exposure to sunlight. Ensure that all vessels are free from oxidizing or reducing compounds.

Store the sodium thiosulfate standard solutions in amber-stained bottles.

9.2 Preparation and titre determination of the 0,01 N sodium thiosulfate standard solution

9.2.1 Preparation of 0,01 N sodium thiosulfate standard solution

See 5.8.

9.2.2 Determination of the titre of the 0,01 N sodium thiosulfate standard solution (factor determination)

Weigh, to the nearest 0,001 mg, 0,27 g to 0,33 g potassium iodate(V) into a volumetric flask [250 ml (6.10) or 500 ml (6.11)] and make up to the mark with water (5.1).

Pipette (6.3) 5 ml or 10 ml of this potassium iodate(V) solution into a 250 ml beaker (6.8). Add 60 ml freshly boiled water (5.1), 5 ml of HCl (5.10) and 0,5 ml of the saturated potassium iodide solution (5.6).

Titrate this solution with the 0,01 N sodium thiosulfate standard solution to determine the factor of the 0,01 N sodium thiosulfate standard solution.

Calculate the factor, f , of the 0,01 N sodium thiosulfate standard solution using Equation (1):

$$f = \frac{m_{\text{KIO}_3} V_1 \times 6 \times 1000 w_{\text{KIO}_3}}{M_{\text{KIO}_3} V_2 V_3 c(\text{Na}_2\text{S}_2\text{O}_3) \times 100} \quad (1)$$

where

- $c(\text{Na}_2\text{S}_2\text{O}_3)$ is the concentration, in moles per litre, of the sodium thiosulfate standard solution (5.8);
- m_{KIO_3} is the mass, in grams, of potassium iodate(V);
- M_{KIO_3} is the relative molecular mass of potassium iodate(V) (214);
- V_1 is the volume, in millilitres, of the potassium iodate(V) solution, used for the titration (5 ml or 10 ml);
- V_2 is the total volume, in millilitres, of the potassium iodate(V) solution (250 ml or 500 ml);
- V_3 is the volume, in millilitres, of the 0,01 N sodium thiosulfate standard solution, used for the determination;
- w_{KIO_3} is the purity, as a mass fraction in grams per 100 g, of the potassium iodate(V);
- 6 is the equivalent mass for the titre (1 mol $\text{KIO}_3 = 3 \text{ mol I}_2$).

9.3 Determination of peroxide value

9.3.1 Purge the carefully cleaned Erlenmeyer flask (6.7) with nitrogen or carbon dioxide. Weigh into the flask, to the nearest 0,1 mg:

- a) either a 5,0 g \pm 0,1 g test portion for expected peroxide values from > 1 to 30;
- b) or a 10,0 g \pm 0,1 g test portion for expected peroxide values from 0 to 1.

The peroxide value is a dynamic value, dependent upon the history of the test sample. Furthermore, the determination of the peroxide value is a highly empirical procedure and the value obtained depends on the mass of the test portion. The user of this International Standard should be aware that due to the prescribed test portion mass, the peroxide values obtained can be slightly lower than those obtained with test portions of lower mass. For some products, the amount of extracted fat/oil can be lower than 5 g, or the peroxide value of the fat/oil can be over 30 meq active oxygen per kilogram. In these cases, the user should choose a smaller test portion mass. As the test portion mass influences the result, report it together with the result.

9.3.2 Dissolve the test portion in 50 ml of the glacial acetic acid/isooctane solution (5.4) by gentle swirling.

In the case of fats with high melting points (hard fats and animal fats), carefully add to the melted fat 20 ml of isooctane (5.3) by gentle swirling, and then immediately add 30 ml of glacial acetic acid (5.2). Also warm the test portion gently, if necessary.

9.3.3 Add the magnetic stirring rod (6.6) and 0,5 ml of the saturated potassium iodide solution (5.6), stir the test portion on the stirrer of the automatic titrator (6.1) for exactly 60 s (use a timer accurate to ± 1 s) at a medium speed to avoid spraying.

9.3.4 Immediately add 30 ml to 100 ml of water (5.1). The amount depends on the apparatus used.

NOTE The greater amount of water is necessary due to phase inversion and depends upon the apparatus used. The phase being titrated is the lower one. With higher amounts of water, the potentiometric difference between the starting and end-point of the titration is bigger (~ 100 mV). This results in a titration curve with a sharp turning point.

9.3.5 Immerse the combined platinum electrode (6.2) into the test sample and start the titration with the 0,01 N sodium thiosulfate standard solution (5.8) while stirring at high speed.

9.3.6 In a parallel blank test, not more than 0,1 ml of the 0,01 N thiosulfate solution shall be used.

9.3.7 Most titration equipment evaluates the equivalent point automatically; otherwise determine the end-point graphically using the point of inflection method.

NOTE Typical end-point titration curves are shown in Figure A.1.

10 Calculation and expression of results

10.1 Calculation

Calculate the peroxide value (PV), in milliequivalents of active oxygen per kilogram, using Formula (2):

$$\frac{(V - V_0)c(\text{Na}_2\text{S}_2\text{O}_3)f \times 1000}{m} \quad (2)$$

where

$c(\text{Na}_2\text{S}_2\text{O}_3)$ is the concentration, in moles per litre, of the 0,01 N sodium thiosulfate standard solution;

f is the factor for the 0,01 N sodium thiosulfate standard solution, determined by Equation (1);

m	is the mass, in grams, of the test portion;
V	is the volume, in millilitres, of the 0,01 N sodium thiosulfate standard solution used for the determination;
V_0	is the volume, in millilitres, of the 0,01 N sodium thiosulfate standard solution used for the blank test.

Report the result of the determination to one decimal place.

11 Precision

11.1 Interlaboratory test

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

11.2 Repeatability

The absolute difference between two independent single test results, obtained with this 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, exceed the repeatability limits, r , given in Table B.1 and Table B.2.

11.3 Reproducibility

The absolute difference between two single test results, obtained with this same method on identical test material in different laboratories by different operators using different equipment, will, in not more than 5 % of cases, exceed the reproducibility limits, R , given in Table B.1 and Table B.2.

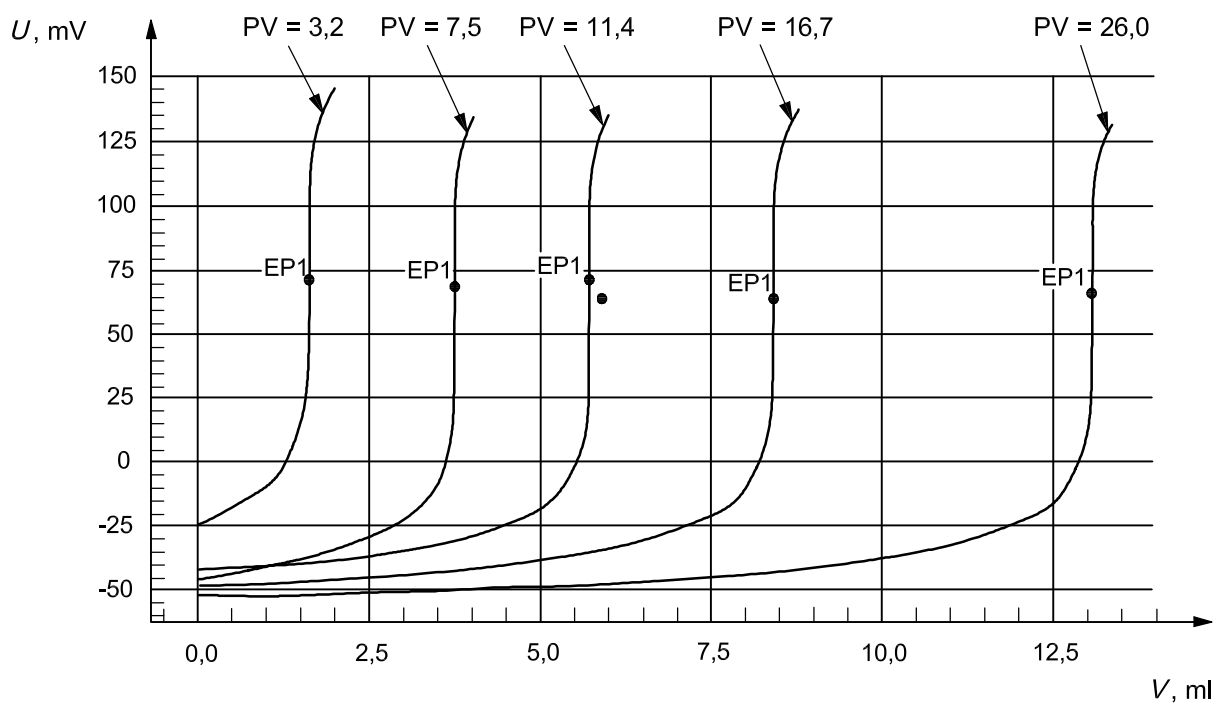
12 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this International Standard;
- d) the mass(es) of the test portion(s);
- e) all operating details not specified in this International Standard or regarded as optional, together with details of any incidents that may have influenced the test result(s);
- f) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

Annex A (informative)

Examples of potentiometric titration curves


Key

EP1 titration end-point
 PV peroxide value
 U potential difference
 V volume

Figure A.1 — Potentiometric titration curves of five samples with different peroxide values

Annex B (informative)

Results of an interlaboratory test

An international collaborative test involving 12 laboratories from five countries (Canada, France, Germany, Iran and Poland) was carried out on the samples listed in Tables B.1 and B.2.

The test was organized by the Deutsches Institut für Normung (DIN) in 2006 and the results obtained were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Tables B.1 and B.2.

Table B.1 — Peroxide values for oils, liquid at room temperature

Sample	Refined oil (A)	Refined sunflower seed oil (B)	Olive oil (D)	Extra virgin olive oil (F)	Extra virgin olive oil (G)	Vegetable oil mixture (I)
No. laboratories participating	12	12	12	12	12	11
No. laboratories after eliminating outliers	12	12	12	11	11	11
No. test results from remaining laboratories	24	24	24	22	22	22
Mean value, meq/kg	0,61	1,27	4,02	13,70	13,13	17,92
Repeatability standard deviation, s_r , meq/kg	0,03	0,06	0,14	0,16	0,25	0,36
Coefficient of variation of repeatability, $CV(r)$, %	5,5	4,4	3,6	1,2	1,9	2,0
Repeatability limit, r ($= 2,8s_r$), meq/kg	0,09	0,16	0,41	0,45	0,71	1,01
Reproducibility standard deviation, s_R , meq/kg	0,11	0,18	0,45	0,82	1,03	1,90
Coefficient of variation of reproducibility, $CV(R)$, %	17,8	14,1	11,3	6,0	7,8	10,6
Reproducibility limit, R ($= 2,8s_R$), meq/kg	0,30	0,50	1,27	2,30	2,87	5,32

Table B.2 — Peroxide values for oils or fats, solid at room temperature

Sample	Lard (C)	Raw palm oil (E)	Palm stearin (H)
No. laboratories participating	12	12	11
No. laboratories after eliminating outliers	12	10	9
No. test results from remaining laboratories	24	20	18
Mean value, meq/kg	1,54	7,52	27,31
Repeatability standard deviation, s_r , meq/kg	0,07	0,15	0,44
Coefficient of variation of repeatability, $CV(r)$, %	4,8	2,0	1,6
Repeatability limit, $r (= 2,8s_r)$, meq/kg	0,21	0,41	1,23
Reproducibility standard deviation, s_R , meq/kg	0,31	0,42	1,78
Coefficient of variation of reproducibility, $CV(R)$, %	20,1	5,6	6,5
Reproducibility limit, $R (= 2,8s_R)$, meq/kg	0,87	1,17	5,00

Bibliography

- [1] ISO 3960, *Animal and vegetable fats and oils — Determination of peroxide value — Iodometric (visual) endpoint determination*
- [2] ISO 3976, *Milk fat — Determination of peroxide value*
- [3] ISO 5555, *Animal and vegetable fats and oils — Sampling*
- [4] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
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- [6] WHEELER, D.H. Peroxide formation as a measure of autoxidative deterioration. *Oil Soap*, 1932, **9**, p. 89-97

