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Animal and vegetable fats and oils — Determination of peroxide value — Iodometric (visual) endpoint determination (ISO 3960:2017)

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National foreword

This British Standard is the UK implementation of EN ISO 3960:2017. It supersedes BS EN ISO 3960:2010 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/307, Oilseeds, animal and vegetable fats and oils and their by-products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Tierische und pflanzliche Fette und Öle - Bestimmung der Peroxidzahl - Iodometrische (visuelle) Endpunktbestimmung (ISO 3960:2017)

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European foreword

This document (EN ISO 3960:2017) has been prepared by Technical Committee ISO/TC 34 “Food products” in collaboration with Technical Committee CEN/TC 307 “Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis” the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2017, and conflicting national standards shall be withdrawn at the latest by August 2017.

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

This fifth edition cancels and replaces the fourth edition (ISO 3960:2007), of which it constitutes a minor revision to exclude fat coming from milk and milk products.

Introduction

Over a period of many years, various methods have been developed for the determination of peroxides in fats and oils. The general principle of most of the methods is the liberation of iodine from potassium iodide in an acid medium. The method according to Wheeler was standardized more than 50 years ago by different standardization bodies, and it is widely used to control commodities by producers, receivers and official laboratories. In national and international food legislation (including the Codex Alimentarius), acceptable limits for the 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 greater than 1, and at 10 g for PV less than or equal to 1, and to limit the applicability of this method to animal and vegetable fats and oils with peroxide values from 0 meq to 30 meq of active oxygen per kilogram. The user of this document 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 — Iodometric (visual) endpoint determination

1 Scope

This document specifies a method for the iodometric determination of the peroxide value of animal and vegetable fats and oils with a visual endpoint detection. The peroxide value is a measure of the amount of oxygen chemically bound to an oil or fat as peroxides, particularly hydroperoxides.

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 (milliequivalents) of active oxygen per kilogram. It is also applicable to margarines and fat spreads with varying water content. The method is not suitable for milk fats and is not applicable to lecithins.

It is to be noted that the peroxide value is a dynamic parameter, whose value is dependent upon the history of the sample. Furthermore, the determination of the peroxide value is a highly empirical procedure and the value obtained depends on the sample mass. It is stressed that, due to the prescribed sample mass, the peroxide values obtained can be slightly lower than those obtained with a lower sample mass.

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this document.

NOTE 1 A preferred method for the iodometric determination of the peroxide value for milk fats is specified in ISO 3976.

NOTE 2 A method for the potentiometric determination of the peroxide value is given in ISO 27107.

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:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp/

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 document

Note 1 to entry: The peroxide value is usually expressed in milliequivalents (meq) of active oxygen per kilogram of oil, but it may also be expressed (in SI units) as millimoles (mmol) of active oxygen per kilogram of oil. The value expressed in millimoles of active oxygen per kilogram is half of that expressed in milliequivalents of active oxygen per kilogram. Multiplication of the peroxide value (meq of active oxygen per kilogram) by the equivalent mass of oxygen (equalling 8) gives the milligrams of active oxygen per kilogram of oil.

4 Principle

The test sample is dissolved in isooctane and glacial acetic acid, and potassium iodide is added. The iodine liberated by the peroxides is determined iodometrically (visually) with a starch indicator and a sodium thiosulfate standard solution. The endpoint of the titration is determined iodometrically (visually).

5 Reagents

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

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

5.1 Water, demineralized, boiled and cooled down to 20 °C.

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

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

5.4 Glacial acetic acid/isooctane solution, prepared by mixing 60 ml of glacial acetic acid and 40 ml of isooctane (volume fraction of glacial acetic acid: φ = 60 ml/100 ml, and volume fraction of isooctane: φ = 40 ml/100 ml).

The mixture is degassed in an ultrasonic bath under vacuum or by purging with a current 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 ρ(KI) = 175 g/100 ml.

Dissolve approximately 14 g of potassium iodide in approximately 8 g of freshly boiled water at room temperature. Make sure the solution remains saturated (undissolved crystals). 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 in 30 ml of the glacial acetic acid/isooctane solution (5.4). If a blue colour is formed and if more than one drop of sodium thiosulfate standard solution (5.7) is needed to remove it, discard the potassium iodide solution.

5.7 0,1 N sodium thiosulfate standard solution, $c(Na_2S_2O_3)$ = 0,1 mol/l.

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

5.8 0,01 N sodium thiosulfate standard solution, $c(Na_2S_2O_3)$ = 0,01 mol/l (see 9.2).

It is necessary to prepare this solution freshly from the 0,1 mol/l sodium thiosulfate standard solution before use or to 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 for the dilution, possibly purged with nitrogen.

5.9 Starch solution, mass concentration ρ = 1 g/100 ml. Mix 0,5 g of starch and a small amount of cold water. Add this mixture, while stirring, to 50 ml of boiling water, boil it for a few seconds and cool immediately.

The solution shall be freshly prepared every day.

It is recommended to use potato starch for iodometry as this starch gives a darker blue colour. Equivalent reagents may also be used.

5.10 Potassium iodate (KIO_3) volumetric standard, secondary reference material, traceable to the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA.

5.11 Hydrochloric acid, c(HCl) = 4 mol/l.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Erlenmeyer flask, of 250 ml capacity, with ground neck and ground glass stopper.

6.2 Burette, of 10 ml or 25 ml capacity, graduated in at least 0,05 ml, preferably with automatic zero adjustment (pellet titrators).

6.3 Manual or automatic dosing unit, of 20 ml capacity, with a resolution of at least 10 µl and an accuracy of ±0,15 % (e.g. a piston burette).

6.4 Pipettes, of 0,5 ml, 1 ml, 10 ml and 100 ml capacity (or automatic pipettes).

6.5 Measuring cylinders, of 50 ml and 100 ml capacity.

6.6 Analytical balance, readable to 0,000 1 g.

6.7 Magnetic stirrer, with magnetic stirring rod (of 2,5 cm) and heating plate.

6.8 Volumetric flask, of 1 000 ml capacity.

6.9 Volumetric flask, of 250 ml capacity.

6.10 Volumetric flask, of 500 ml capacity.

6.11 Microwave oven.

A microwave oven may be used to melt solid samples in an easy and quick manner. Careful and proper use of a microwave oven will not lead to any increase in the peroxide value. The suitable conditions shall be tested in advance.

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 document. A recommended sampling method is given in ISO 5555.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

The test sample for the determination of peroxide value shall be taken first and the peroxide value shall be determined immediately.

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

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 of active oxygen per kilogram. In these cases, the user should choose a smaller sample mass [see Clause 12 f)].

9 Procedure

9.1 General

Carry out all steps in diffuse daylight or in artificial light. Avoid direct exposure to sunlight. Observe 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 the 0,01 N sodium thiosulfate standard solution

By means of a pipette (6.4), transfer 100 ml of the 0,1 N sodium thiosulfate standard solution (5.7) to a volumetric flask of 1 000 ml capacity (6.8). Dilute to the mark with freshly boiled water (5.1). After homogenization, transfer the obtained 0,01 N sodium thiosulfate standard solution to an amber-stained bottle.

Prepare daily the 0,01 N sodium thiosulfate standard solution freshly from the 0,1 N sodium thiosulfate standard solution before use, or determine the titre. 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 for the dilution, possibly purged with nitrogen.

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

Weigh, to the nearest 0,001 g, 0,27 g to 0,33 g of potassium iodate (KIO_3) into a volumetric flask (250 ml or 500 ml) (6.9 or 6.10) and dilute to the mark with freshly boiled water (5.1), cooled down to room temperature.

By means of a pipette (6.4), transfer 5 ml or 10 ml of this potassium iodate solution into a 250 ml Erlenmeyer flask (6.1). Add 60 ml of freshly boiled water, 5 ml of 4 mol/l hydrochloric acid and 25 mg to 50 mg of potassium iodide (5.5) or 0,5 ml of the saturated potassium iodide solution (5.6).

Titrate this solution using the iodometric (visual) method to determine the factor of the 0,01 N sodium thiosulfate standard solution (see 9.2.1).

Calculate the factor, *F*, of the 0,01 N sodium thiosulfate solution using Formula (1):

$$F = \frac{m_{KIO_3} \cdot V_1 \cdot 6 \cdot 1\,000 \cdot w_{KIO_3}}{M_{KIO_3} \cdot V_2 \cdot V_3 \cdot c_{thio} \cdot 100} \quad (1)$$

where

6 is the equivalent mass for the titre (1 mol $KIO_3 \Leftrightarrow$ 3 mol I_2);

V_1 is the volume of the potassium iodate solution, used for the titre determination (5 ml or 10 ml);

V_2 is the total volume of potassium iodate solution, in millilitres (250 ml or 500 ml);

V_3 is the volume of 0,01 N thiosulfate solution used for the determination, in millilitres;

m_{KIO3} is the mass of potassium iodate, in grams;

w_{KIO3} is the purity of potassium iodate, in g/100 g;

M_{KIO3} is the molecular mass of potassium iodate (214 g/mol);

c_{thio} is the concentration of the sodium thiosulfate standard solution, in moles per litre (0,01 mol/l).

9.3 Determination of peroxide value

9.3.1 Rinse the carefully cleaned Erlenmeyer flask (6.1) with nitrogen or carbon dioxide. Weigh the following into the flask, to the nearest 0,1 mg:

a) 5,0 g ± 0,1 g of test sample for expected peroxide values from >1 to 30;

b) 10,0 g ± 0,1 g of test sample for expected peroxide values from 0 to 1.

Rinse the Erlenmeyer flask with the glacial acetic acid/isooctane solution (5.4) prior to use to ensure that the flask does not contain any oxidizing or reducing substances.

9.3.2 Dissolve the test sample in 50 ml of the glacial acetic acid/isooctane solution 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 sample gently, where necessary.

9.3.3 Add 0,5 ml of the saturated potassium iodide solution (5.6). Close the Erlenmeyer flask (6.1) and mix with a magnetic stirrer (6.7) without creating a large vortex, or manually without aeration for exactly 60 s (use a timer accurate to ±1 s).

9.3.4 Open the Erlenmeyer flask (6.1), immediately add 100 ml of demineralized water, rinse the ground glass stopper and swirl.

9.3.5 Immediately titrate the liberated iodine with the 0,01 N sodium thiosulfate standard solution (5.8) from yellow orange to pale yellow and, after addition of 0,5 ml of starch solution (5.9), from violet to colourless. Stop the titration as soon as the solution is colourless for 30 s.

NOTE 1 The phase being titrated is the lower one. There is a delay of 15 s to 30 s in the change of colour with the 0,01 N sodium thiosulfate standard solution (5.8).

NOTE 2 In the case of peroxide values below 1, the starch solution can be added at the beginning of the titration.

9.3.6 In a parallel blank test, not more than 0,1 ml of the 0,01 N thiosulfate solution shall be used. If the blank test is higher, then replace the saturated potassium iodide solution as it could be unsuitable.

10 Calculation and expression of results

Calculate the peroxide value (commonly known in the industry as “PV”), in milliequivalents (meq) of active oxygen per kilogram, using Formula (2):

$$\frac{(V - V_0) \cdot c_{\text{thio}} \cdot F \cdot 1\,000}{m} \tag{2}$$

where

V is the volume of sodium thiosulfate solution used for the determination, in millilitres;

V_0 is the volume of the sodium thiosulfate standard solution used for the blank test, in millilitres;

c_{thio} is the concentration of the sodium thiosulfate solution, in moles per litre;

m is the mass of the test portion, in grams;

F is the factor of the 0,01 N sodium thiosulfate solution, determined according to 9.2.

The result of the determination shall be reported to one decimal place.

11 Precision of the method

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. 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 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 values of r given in Tables A.1 and A.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 values of *R* given in Tables A.1 and A.2.

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;

c) the test method used, with reference to this document, i.e. ISO 3960;

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

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

f) whether or not the user has chosen a smaller sample mass.

As the sample mass influences the result, this shall be reported together with the result.

Annex A
(informative)

Results of an interlaboratory test

An international collaborative test involving 23 laboratories in nine countries was carried out on the following samples.

A: Refined sunflower/rape-seed oil (1:1)

B: Olive oil (mixture of refined and virgin olive oil)

C: Extra virgin olive oil

D: Extra virgin olive oil

E: Rape-seed oil, aged

F: Lampante olive oil

G: Tallow

H: Lard

I: Palm oil

J: Palm stearin

K: Coconut oil

The test was organized by the Deutsches Institut für Normung (DIN) in 2004/2005. 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 Table A.1.

Table A.1 — Test on oils that are liquid at room temperature

	Sample					
	A	**B**	**C**	**D**	**E**	**F**
Number of laboratories participating	23	23	21	23	23	23
Number of laboratories after eliminating outliers	21	21	18	22	23	22
Number of test results from remaining laboratories	42	42	36	44	46	44
Mean value, meq/kg	1,63	3,21	8,34	12,04	19,02	26,92
Repeatability standard deviation, s_r, meq/kg	0,10	0,08	0,25	0,26	0,36	0,33
Repeatability relative standard deviation, %	6,0	2,6	3,0	2,2	1,9	1,2
Repeatability limit, r (= 2,8 s_r), meq/kg	0,27	0,23	0,69	0,73	1,01	0,92
Reproducibility standard deviation, s_R, meq/kg	0,22	0,46	0,80	1,07	1,71	3,06
Reproducibility relative standard deviation, %	13,3	14,2	9,6	8,9	9,0	11,4
Reproducibility limit, R (= 2,8 s_R), meq/kg	0,61	1,28	2,25	3,00	4,78	8,57

Table A.2 — Test on fats that are solid at room temperature

	Sample					
	G	**H**	**I**	**J**	**K** (5 g)	**K** (10 g)
Number of laboratories participating	16	16	16	16	16	16
Number of laboratories after eliminating outliers	15	15	14	12	13	11
Number of test results from remaining laboratories	30	30	28	24	26	22
Mean value, meq/kg	1,60	3,67	2,99	4,77	0,55	0,71
Repeatability standard deviation, s_r, meq/kg	0,07	0,09	0,08	0,17	0,06	0,04
Repeatability relative standard deviation, %	4,6	2,3	2,7	3,66	11,4	6,0
Repeatability limit, r (= 2,8 s_r), meq/kg	0,20	0,24	0,22	0,49	0,17	0,12
Reproducibility standard deviation, s_R, meq/kg	0,45	0,48	0,44	0,27	0,19	0,25
Reproducibility relative standard deviation, %	28,0	13,0	14,7	5,6	34,7	34,8
Reproducibility limit, R (= 2,8 s_R), meq/kg	1,25	1,33	1,23	0,75	0,53	0,69

Bibliography

[1] ISO 3976, *Milk fat — Determination of peroxide value*

[2] ISO 5555, *Animal and vegetable fats and oils — Sampling*

[3] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] 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*

[5] ISO 27107, *Animal and vegetable fats and oils — Determination of peroxide value — Potentiometric end-point determination*

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