



## Method of Analysis

# Determination of the Peroxide Value

### 1. Scope and Field of Application

This standard describes the method for the determination of the peroxide value of animal and vegetable oils and fats with titrimetric or potentiometric endpoint detection.

### 2. Definition

The peroxide value is the quantity of those substances in the sample, expressed in terms of milliequivalents of active oxygen per kilogram, which oxidize potassium iodide under the operating conditions described.

### 3. Principle

Treatment of the test portion, dissolved in isooctane and glacial acetic acid, with a solution of potassium iodide. Titration of the liberated iodine with standardized sodium thiosulfate solution. The endpoint of the titration is determined iodometrically (visually) or electrochemically.

### 4. Reagents

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

**4.1** Water, demineralized, boiled and cooled.

**4.2** 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).

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

**4.4** Glacial acetic acid/isooctane solution, prepared by mixing 60 ml of glacial acetic acid (4.2) and 40 ml of isooctane (4.3) (volume fraction of glacial acetic acid:  $\phi = 60 \text{ ml}/100 \text{ ml}$ , and volume fraction of isooctane:  $\phi = 40 \text{ ml}/100 \text{ ml}$ ).

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).

**4.5** Potassium iodide, free from iodine and iodates.

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

Dissolve approximately 14 g of potassium iodide (4.5) 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 (4.9) to 0,5 ml of the potassium iodide in 30 ml of the glacial acetic acid/isooctane solution (4.4). If a blue colour is formed and if more than one drop of sodium thiosulfate standard solution (4.7) is needed to remove it, discard the potassium iodide solution.

**4.7** 0,1 N sodium thiosulfate standard solution,  $c(\text{Na}_2\text{S}_2\text{O}_3) = 0,1 \text{ mol}/\text{l}$ .

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 (5.10).

**4.8** 0,01 N sodium thiosulfate standard solution,  $c(\text{Na}_2\text{S}_2\text{O}_3) = 0,01 \text{ mol}/\text{l}$ .

As an example, pipette (5.4) 100 ml of the 0,1 N sodium thiosulfate standard solution (4.7) into a volumetric flask of capacity 1 000 ml (5.8) (1/10 dilution). Make up to the mark with water (4.1) (volumes can be adapted regarding number of samples to be quantified; hence, use other volumetric flasks (5.8)). After homogenization, transfer the obtained 0,01 N sodium thiosulfate standard solution to an amber-stained bottle (5.10).

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 titer 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 (4.1) for the dilution, possibly purged with nitrogen.

The following procedure is recommended to determine the titer 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 potassium iodate (4.10) into a volumetric flask [250 ml (5.8) or 500 ml (5.8)] and make up to the mark with water (4.1).

Pipette (5.4) 5 ml or 10 ml of this potassium iodate solution into a 250 ml Erlenmeyer flask (5.9). Add 60 ml freshly boiled water (4.1), 5 ml of HCl (4.11) and 0.5 ml of the saturated potassium iodide solution (4.6).

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

Calculate the factor, F, of the 0,01 N sodium thiosulfate solution using the following formula:

$$F = \frac{m_{\text{KIO}_3} \cdot V_1 \cdot 6 \cdot 1000 \cdot w_{\text{KIO}_3}}{M_{\text{KIO}_3} \cdot V_2 \cdot V_3 \cdot c_{\text{thio}} \cdot 100} \quad (1)$$

Where:

- 6 is the equivalent mass for the titer ( $1 \text{ mol KIO}_3 \Leftrightarrow 3 \text{ mol I}_2$ );
- $V_1$  is the volume of the potassium iodate solution used for the titer determination (5 ml or 10 ml);
- $V_2$  is the total volume of potassium iodate solution, in milliliters (250 ml or 500 ml);
- $V_3$  is the volume of 0,01 N thiosulfate solution used for the determination, in milliliters;
- $m_{\text{KIO}_3}$  is the mass of potassium iodate, in grams;
- $w_{\text{KIO}_3}$  is the purity of potassium iodate, in g/100 g;
- $M_{\text{KIO}_3}$  is the molecular mass of potassium iodate (214 g/mol);
- $c_{\text{thio}}$  is the concentration of the sodium thiosulfate standard solution, in moles per litre (0,01 mol/l).

**4.9** Starch solution, mass concentration  $\rho = 1 \text{ g}/100 \text{ ml}$  (10 g/l) aqueous dispersion. 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.

**4.10** Potassium iodate ( $\text{KIO}_3$ ) volumetric standard, secondary reference material, traceable to the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA.

**4.11** Hydrochloric acid,  $c(\text{HCl}) = 4 \text{ mol/l}$ .

## 5. Apparatus

All the equipment used shall be free from reducing or oxidizing substances.

*NOTE Do not grease ground surfaces.*

- 5.1** Erlenmeyer flask, of 250 ml capacity, with ground neck and ground glass stopper.
- 5.2** Burette, of 5 ml, 10 ml or 25 ml capacity, graduated in at least 0,05 ml, preferably with automatic zero adjustment (pellet titrators).
- 5.3** Manual or automatic dosing unit, of 20 ml capacity (other volumes can be accepted), with a resolution of at least 10  $\mu\text{l}$  and an accuracy of  $\pm 0,15 \%$  (e.g. a piston burette).

- 5.4 Pipettes, of 0,5 ml, 1 ml, 10 ml, 20 mL and 100 ml capacity, or others in order to implement an 1/10 dilution (or automatic pipettes).
- 5.5 Measuring cylinders, of 50 ml and 100 ml capacity.
- 5.6 Analytical balance, readable to 0,000 1 g.
- 5.7 Magnetic stirrer, with magnetic stirring rod and heating plate.
- 5.8 Volumetric flask, of 1 000 ml, 500 ml, 250 ml, 200 ml and 100 ml capacity (depending on the prepared volume).
- 5.9 Erlenmeyer or beaker, of capacity 250 ml, tall form.
- 5.10 Amber-stained bottles, of 1 000 ml capacity (or others depending on the prepared volume: 100 ml, 200 ml, 250 ml, 500 ml...).
- 5.11 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 endpoint). This is necessary to minimize the titration time whilst achieving a slow titration near the endpoint.

5.12 Combined platinum electrode.

5.13 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.

## 6. Sample Preparation

Take care that the sample is taken and stored away from the light (daylight or artificial light), kept cold and contained in completely filled glass containers, hermetically sealed with ground-glass or cork stoppers.

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. Test samples with visible impurities shall be filtered.

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.

## 7. Procedure

It is advisable to rinse the Erlenmeyer flask or the beaker with the glacial acetic acid/isooctane solution (4.4) prior to use, in order to ensure that the flask does not contain any oxidising or reducing substances. Let dry and purge the Erlenmeyer flask (5.1) for iodometric determination or the beaker (5.9) for potentiometric determination with nitrogen or carbon dioxide. Weigh the following into the flask, to the nearest 0,1 mg:

- a) 5,0 g  $\pm$  0,1 g of test sample for expected peroxide values from >1 to 30;
- b) 10,0 g  $\pm$  0,1 g of test sample for expected peroxide values from 0 to 1.

The peroxide value of the fat/oil can be over 30 meq active oxygen per kilogram. In this case, the user should choose a smaller test portion mass.

Then, to determine the peroxide value, use procedure 7.1 for iodometric determination (visually) or use procedure 7.2 for potentiometric determination.

## 7.1. Iodometric determination of peroxide value

**7.1.2** 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 (4.3) by gentle swirling, and then immediately add 30 ml of glacial acetic acid (4.2). Also warm the test sample gently, where necessary.

**7.1.3** Add 0,5 ml of the saturated potassium iodide solution (4.6). Close the Erlenmeyer flask (5.1) and mix with a magnetic stirrer (5.7) without creating a large vortex, or manually without aeration for exactly 60 s (use a timer accurate to  $\pm 1$  s).

**7.1.4** Open the Erlenmeyer flask (5.1), immediately add 100 ml of demineralized water, rinse the ground glass stopper and swirl.

**7.1.5** Immediately titrate the liberated iodine with the 0,01 N sodium thiosulfate standard solution (4.8) from yellow-orange to pale yellow and, after addition of 0,5 ml of starch solution (4.9), 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 (4.8).

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

NOTE 3 The determination may cause an emulsion when water is added. To avoid this emulsion, a moderate circular agitation (always in the same direction) is recommended during the titration.

**7.1.6** In the 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, for example, the saturated potassium iodide solution, as it could be unsuitable, or the acetic acid or one of the other reagents.

## **7.2. Potentiometric determination of peroxide value**

**7.2.1** Dissolve the test portion in 50 ml of the glacial acetic acid/isooctane solution (4.4) by gentle swirling.

**7.2.2** Add the magnetic stirring rod (5.7) and 0,5 ml of the saturated potassium iodide solution (4.6), stir the test portion on the stirrer of the automatic titrator (5.11) for exactly 60 s (use a timer accurate to  $\pm 1$  s) at a medium speed to avoid spraying.

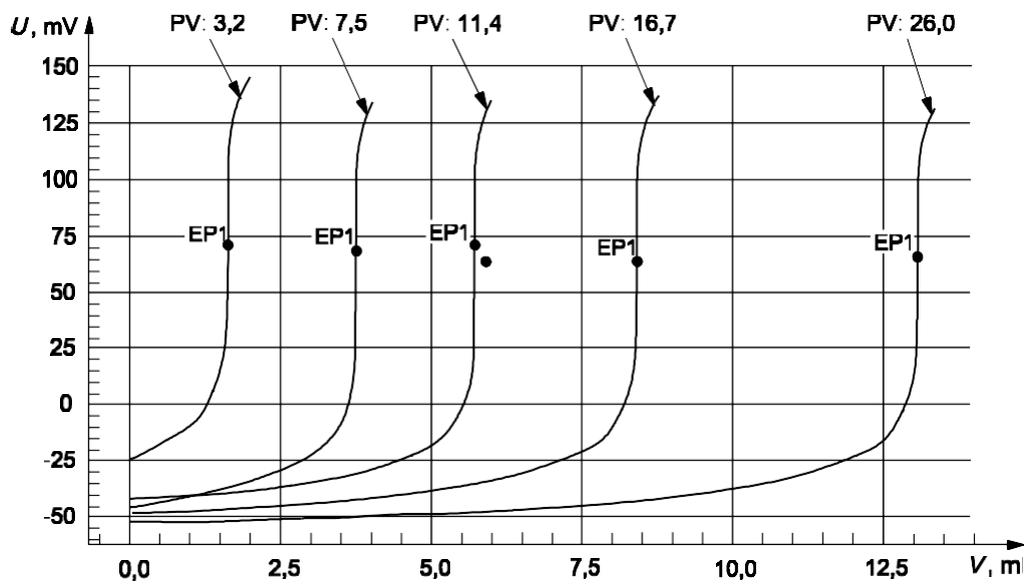
**7.2.3** Immediately add 30 ml to 100 ml of water (4.1). The amount depends on the apparatus used.

**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 ( $\sim 100$  mV). This results in a titration curve with a sharp turning point.

**7.2.4** Immerse the combined platinum electrode (5.12) into the test sample and start the titration with the 0,01 N sodium thiosulfate standard solution (4.8) while stirring at high speed.

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

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



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

Figure 1: Potentiometric titration curves of five samples with different peroxide values

## 8. Calculation and Expression of Results

The peroxide value (PV), expressed in milliequivalents of active oxygen per kilogram, is given by the following formula (2):

$$\frac{(V - V_0) \cdot c_{\text{thio}} \cdot F \cdot 1000}{m} \quad (2)$$

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;
- $c_{\text{thio}}$  is the concentration of the sodium thiosulfate solution, in moles per liter;
- $m$  is the mass of the test portion, in grams;
- $F$  is the factor of the 0,01 N sodium thiosulfate solution, determined according to 4.8.

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

**Precision Values of the Method**  
(Data from ISO 3960 and ISO 27107 standards)

**1. Results of the interlaboratory test for iodometric determination (ISO 3960)**

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)	G: Tallow
B: Olive oil (mixture of refined and virgin olive oil)	H: Lard
C: Extra virgin olive oil	I: Palm oil
D: Extra virgin olive oil	J: Palm stearin
E: Rape-seed oil, aged	K: Coconut oil
F: Lampante olive 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 Tables 1 and 2.

**Table 1:** Peroxide values 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 2:** Peroxide values on oils 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

## 2. Results of the interlaboratory test for potentiometric determination (ISO 27107)

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 3 and 4.

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 3 and 4.

**Table 3:** Peroxide values on oils that are 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)
Number of laboratories participating	12	12	12	12	12	11
Number of laboratories after eliminating outliers	12	12	12	11	11	11
Number of 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						
Coefficient of variation of repeatability, $CV(r)$ , %	0,03	0,06	0,14	0,16	0,25	0,36
Repeatability limit, $r (= 2,8s_r)$ , meq/kg	5,5	4,4	3,6	1,2	1,9	2,0
	0,09	0,16	0,41	0,45	0,71	1,01
Reproducibility standard deviation, $s_R$ , meq/kg						
Coefficient of variation of reproducibility, $CV(R)$ , %	0,11	0,18	0,45	0,82	1,03	1,90
Reproducibility limit, $R (= 2,8s_R)$ , meq/kg	17,8	14,1	11,3	6,0	7,8	10,6
	0,30	0,50	1,27	2,30	2,87	5,32

**Table 4:** Peroxide values on oils that are solid at room temperature

Sample	Lard (C)	Raw palm oil (E)	Palm stearin (H)
Number of laboratories participating	12	12	11
Number of laboratories after eliminating outliers	12	10	9
Number of 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

### 3. References

ISO 5725-1:1994 Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions

ISO 5725-2:1994 Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of the repeatability and reproducibility of a standard measurement method

ISO 5725-5:1998 Accuracy (trueness and precision) of measurement methods and results – Part 5: Alternative methods for the determination of the precision of a standard measurement method

ISO 5725-6:1994 Accuracy (trueness and precision) of measurement methods and results – Part 6: Use in practice of accuracy values

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

ISO 27107:2008 Animal and vegetable fats and oils — Determination of peroxide value — Potentiometric end-point determination