INTERNATIONAL STANDARD

ISO 3960

Second edition
1998-08-15

Animal and vegetable fats and oils — Determination of peroxide value

Corps gras d'origines animale et végétale — Détermination de l'indice de peroxyde

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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.

International Standard ISO 3960 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* Subcommittee SC 11, *Animal and vegetable fats and oils.*

This second edition cancels and replaces the first edition (ISO 3960:1977), which has been technically revised.

Annexes A and B of this International Standard are for information only.

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

Printed in Switzerland

Animal and vegetable fats and oils — Determination of peroxide value

1 Scope

This International Standard specifies a method for the determination of the peroxide value of animal and vegetable oils and fats.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication the edition indicated was valid. All standards are subject to revision, and parties to agreement based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

3 Definition

For the purposes of this International Standard, the following definition applies.

3.1
peroxide value
quantity of those substances in the sample, expressed in terms of active oxygen, which oxidize potassium iodide under the conditions specified in this International Standard, divided by the mass of the test portion

NOTE 1 Peroxide value is expressed in millimoles per kilogram.

NOTE 2 Peroxide value is often expressed in industry as milliequivalents per kilogram. The value expressed in millimoles per kilogram is half that expressed in milliequivalents per kilogram (see clause 10).

4 Principle

Treatment of a test portion, in solution in acetic acid and iso-octane, with a solution of potassium iodide. Titration of the liberated iodine with a standard volumetric sodium thiosulfate solution.

5 Reagents

Use only reagents of recognised analytical grade, unless otherwise stated. All reagents and the water shall be free of dissolved oxygen.

5.1 Water, complying with grade 3 of ISO 3696.

5.2 Glacial acetic acid, freed from oxygen by purging with a current of pure and dry inert gas (carbon dioxide or nitrogen).

WARNING: Glacial acetic acid is moderately toxic by ingestation and inhalation. It is a strong irritant to skin and tissue.

5.3 Iso-octane, freed from oxygen by purging with a current of pure and dry inert gas (carbon dioxide or nitrogen).

WARNING: Iso-octane is flammable and a fire risk. Explosive limits in air are 1,1 % (*V/V*) to 6,0 % (*V/V*). It is toxic by ingestation and inhalation. A properly operating fume hood should be used when working with this solvent.

5.4 Acetic acid/iso-octane solution [60:40 (*V/V*)], prepared by mixing 3 volumes of glacial acetic acid (5.2) with 2 volumes of iso-octane (5.3).

5.5 Potassium iodide solution, saturated, recently prepared and free from free iodine and iodates.

Make sure the solution remains saturated as indicated by the presence of undissolved crystals. Store in the dark. Test daily by adding 2 drops of starch solution (5.9) to 0,5 ml of the potassium iodide solution in 30 ml of the acetic acid/iso-octane solution (5.4). If a blue colour is formed which requires more than 1 drop of sodium thiosulfate solution (5.7) to discharge the colour, discard the potassium iodide solution and prepare a fresh solution.

5.6 Sodium thiosulfate solution, $c(Na_2S_2O_3)$ = 0,1 mol/l, standardized just before use.

Dissolve 24,9 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$) in distilled water and dilute to 1 litre.

5.7 Sodium thiosulfate solution, $c(Na_2S_2O_3)$ = 0,01 mol/l, standardized just before use.

Prepare by diluting solution 5.6.

5.8 Sodium thiosulfate solution, $c(Na_2S_2O_3)$ = 0,002 mol/l, standardized just before use.

Prepare by diluting solution 5.6.

5.9 Starch solution, 5 g/l.

Mix 1 g of starch and a small amount of cold distilled water. Add this mixture, while stirring, to 200 ml of boiling water. Add 250 mg of salicylic acid as preservative and boil for 3 min. Immediately remove from the heat and cool.

If long storage is required, keep the solution in a refrigerator at between 4 °C and 10 °C. A fresh starch solution shall be prepared when the endpoint of the titration from blue to colourless fails to be sharp. If stored under refrigeration, the starch solution should be stable for about 2 to 3 weeks.

NOTE The sensitivity of the starch solution may be tested as follows. To 5 ml of the starch solution (5.9) in 100 ml of water, add 0,05 % of potassium iodide (5.5) solution and 1 drop of 0,05 % sodium hypochlorite solution. It is essential that the deep blue colour produced be discharged by 0,05 ml of sodium thiosulfate solution (5.6).

6 Apparatus

All equipment used shall be free from reducing or oxidizing substances. Do not grease ground glass surfaces.

Usual laboratory apparatus and, in particular, the following.

6.1 Conical flasks , of 250 ml capacity, with ground glass stoppers.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555 (see reference [1]).

It is important that the laboratory receive a sample that is truly representative and has not been damaged or changed during transport or storage.

Ensure that the sample is taken and stored away from strong light, kept cold, and contained in completely filled glass containers, hermetically sealed with ground glass stoppers.

8 Preparation of test sample

Ensure that the packaging of the fat or oil is not damaged, and is well closed. When parameters other than the peroxide value have to be investigated, the test portion for the peroxide value shall be taken first from the laboratory sample.

Do not melt solid fats, but take a part of the fat from the middle of the material, thus preventing fat from the surface getting into the test portion. Immediately transfer the test portion to a conical flask and stopper the flask.

Partly solid samples shall be homogenized by mixing and if necessary gentle warming, taking care to exclude air from the sample. The test portion should be taken from the middle of the sample.

For oils, pipette the test portion from the middle of the sample.

9 Procedure

Carry out the test in artificial light or diffuse daylight.

NOTE If it is required to check that the repeatability requirement (see 11.2) is met, carry out two single determinations in accordance with 9.1 and 9.2.

9.1 Test portion

Rinse the conical flask (6.1) with a current of pure dry inert gas (carbon dioxide or nitrogen). Into the flask, weigh a mass of the sample to the accuracy given in table 1 and in accordance with the expected peroxide value.

Table 1 — Mass of test portion and accuracy of weighing

Expected peroxide value mmol/kg	Mass of test portion g	Weighing accuracy g
0 to 6	5,0 to 2,0	±0,01
6 to 10	2,0 to 1,2	±0,01
10 to 15	1,2 to 0,8	±0,01
15 to 25	0,8 to 0,5	±0,001
25 to 45	0,5 to 0,3	±0,001

9.2 Determination

Add 50 ml of acetic acid/iso-octane solution (5.4) to the conical flask and replace the stopper. Swirl the flask until the sample has dissolved. Using a suitable volumetric pipette, add 0,5 ml of saturated potassium iodide solution (5.5) and replace the stopper. Allow the solution to react for 1 min ± 1 s, thoroughly shaking it at least three times during this period, then immediately add 30 ml of distilled water.

Titrate the solution with sodium thiosulfate solution (5.7), adding it gradually and with constant, vigorous agitation, until the yellow iodine colour has almost disappeared. Add about 0,5 ml of starch solution (5.9) and continue the titration with constant agitation, especially near the endpoint, to liberate all of the iodine from the solvent layer, adding the sodium thiosulfate solution drop by drop until the blue colour just disappears.

If the titration uses less than 0,5 ml of 0,01 mol/l sodium thiosulfate solution (5.7), repeat the determination using the 0,002 mol/l sodium thiosulfate solution (5.8) with vigorous constant agitation.

NOTE There is a 15 s to 30 s delay in neutralizing the starch indicator for peroxide values of 35 mmol/kg and greater, due to the tendency of iso-octane to float on the surface of the aqueous medium and the time necessary to mix adequately the solvent and the aqueous titrant, thus liberating the last traces of iodine. Based on interlaboratory test results, it is recommended to use the 0,01 mol/l sodium thiosulfate solution (5.7) for peroxide values below 35 mmol/kg and the 0,002 mol/l sodium thiosulfate solution (5.8) for peroxide values equal to or less than 6 mmol/kg. A small amount [0,5 % to 1,0 % (*m/m*)] of a high HLB emulsifier (e.g. Tween 60)[1)] may be added to the reaction mixture to retard the phase separation and decrease the time lag in the liberation of iodine.

9.3 Blank determination

Carry out a blank determination in parallel with the sample determination. If the result of the blank determination exceeds 0,05 ml of 0,002 mol/l sodium thiosulfate solution (5.8), replace the impure reagents and repeat the sample determination.

10 Expression of results

Calculate the peroxide value **expressed in millimoles of active oxygen per kilogram**, P, by the following equation:

$$P = \frac{1000\,(V - V_0)\,c}{2m}$$

where

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

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

c is the concentration of the sodium thiosulfate solution, in moles per litre;

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

NOTE The peroxide value **expressed in milliequivalents per kilogram**, P_e, may be calculated, if required, from the following equation:

$$P_e = \frac{1000\,(V - V_0)\,c}{m}$$

1) Tween 60 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

11 Precision

11.1 Interlaboratory tests

Details of interlaboratory tests on the precision of the method are summarized in annex A. The values derived from these interlaboratory tests may not be applicable to concentrations 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 10 % of the mean of the two results, for peroxide values less than or equal to 20 mmol/kg.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases be greater than 75 % of the mean of the two results, for peroxide values less than or equal to 20 mmol/kg.

12 Test report

The test report shall specify

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

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

— the test result(s) obtained, and **the units in which the result is expressed**;

— if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory tests

International collaborative tests have been carried out on the method given in this International Standard in accordance with ISO 5725[2)].

A test on sunflowerseed oil was organized by the Federation of Oils, Seeds and Fats Associations (FOSFA) in 1993. The results are given in table A.1.

Table A.1 — Test on sunflowerseed oil

	Sample 36A	Sample 36B
Number of laboratories	33	33
Number of acceptable results	33	33
Mean value, mmol/kg	18,1	18,9
Repeatability limit (r), mmol/kg	1,3	1,0
Reproducibility limit (R), mmol/kg	15,6	15,6

A test on lard, tallow and beef fat was organized by the United Kingdom in 1994. The results are given in table A.2.

Table A.2 — Test on lard, tallow and beef fat

	Lard	Tallow	Beef fat
Number of laboratories	11	11	11
Number of acceptable results	11	11	11
Mean value, mmol/kg	5,2	7,0	6,1
Repeatability limit (r), mmol/kg	0,4	1,1	0,5
Reproducibility limit (R), mmol/kg	2,4	5,7	5,8

2) ISO 5725:1986 (now withdrawn) was used to obtain the precision data.

Annex B
(informative)

Bibliography

[1] ISO 555:1991, *Animal and vegetable fats and oils — Sampling.*

[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

[3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.*

[4] ISO 5725-2:1994, *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.*