BS EN ISO 3961:2013

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# Animal and vegetable fats and oils — Determination of iodine value (ISO 3961:2013)

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**National foreword**

This British Standard is the UK implementation of EN ISO 3961:2013. It supersedes BS EN ISO 3961:2011 which is withdrawn.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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ISBN 978 0 580 79271 7

ICS 67.200.10

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 July 2013.

**Amendments issued since publication**

| Date | Text affected |
|---|---|

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

# EN ISO 3961

July 2013

ICS 67.200.10

Supersedes EN ISO 3961:2011

English Version

# Animal and vegetable fats and oils - Determination of iodine value (ISO 3961:2013)

Corps gras d'origines animale et végétale - Détermination de l'indice d'iode (ISO 3961:2013)

Tierische und pflanzliche Fette und Öle - Bestimmung der Iodzahl (ISO 3961:2013)

This European Standard was approved by CEN on 5 July 2013.

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Ref. No. EN ISO 3961:2013: E

# Foreword

This document (EN ISO 3961:2013) 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of an identical text or by endorsement, at the latest by January 2014, and conflicting national standards shall be withdrawn at the latest by January 2014.

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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 fifth edition cancels and replaces the fourth edition (ISO 3961:2009), which has been technically revised.

# Animal and vegetable fats and oils — Determination of iodine value

## 1 Scope

This International Standard specifies a reference method for the determination of the iodine value (commonly known in the industry as IV) of animal and vegetable fats and oils, hereinafter referred to as fats.

Annex B describes a method for the calculation of the IV from fatty acid compositional data. This method is not applicable to fish oils. Furthermore cold-pressed, crude and unrefined vegetable oils as well as (partially) hydrogenated oils can give different results by the two methods. The calculated IV is affected by impurities and thermal degradation products.

NOTE The method in Annex B is based upon the AOCS Official method Cd 1c-85.[9]

## 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**
**iodine value**
**IV**
mass of halogen, expressed as iodine, absorbed by the test portion following the specified procedure, divided by the mass of the test portion

Note 1 to entry: The IV is expressed as a mass fraction in grams per 100 g of fat.

## 4 Principle

Dissolution of a test portion in solvent and addition of Wijs reagent. After a specified time, addition of potassium iodide and water, and titration of the liberated iodine with sodium thiosulfate solution.

NOTE Annex B describes a method for the calculation of the IV from fatty acid compositional data. However, this is not intended to be a rapid method. The method gives two results from one analytical procedure. The volumetric method is the reference method.

## 5 Reagents

Use only reagents of recognized analytical grade.

**WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed. Wijs solution causes severe burns; vapours can cause lung and eye damage. A fume hood shall be used for the work.**

**5.1 Water**, in accordance with ISO 3696,[4] grade 3.

**5.2 Potassium iodide** solution, mass concentration, $\rho$(KI) = 100 g/l, not containing iodate or free iodine.

**5.3 Starch solution**. Mix 5 g of soluble starch in 30 ml of water (5.1) and add to 1 000 ml of boiling water. Boil for 3 min and allow to cool. Prepare fresh starch solution every day.

**5.4 Sodium thiosulfate**, standard volumetric solution, amount of substance concentration $c(Na_2S_2O_3 \cdot 5H_2O)$ = 0,1 mol/l, standardized not more than 7 days before use.

**5.5 Solvent**, prepared by mixing one volume of cyclohexane (50 ml) and one volume of glacial acetic acid (50 ml), volume fractions $\varphi$ = 50 ml/100 ml.

**5.6 Wijs reagent**, containing iodine monochloride in acetic acid. The I/Cl ratio of the Wijs reagent shall be within the limits 1,10 ± 0,1. Wijs reagent is sensitive to temperature, moisture, and light. Store in the dark at <30 °C.

Use commercially available Wijs reagent. Observe any shelf-life limitation of the reagent.

## 6 Apparatus

Usual laboratory apparatus and, in particular, the following.

**6.1 Glass weighing scoops**, suitable for the test portion and for insertion into the flasks (6.2).

**6.2 Conical flasks**, capacity 500 ml, fitted with ground glass stoppers and showing no evidence of the presence of moisture.

**6.3 Analytical balance**, readability 0,000 1 g and weighing accuracy 0,001 g.

**6.4 Volumetric flask**, capacity 1 000 ml, ISO 1042,[3] class A.

**6.5 Pipette**, capacity 25 ml, automatic, ISO 8655-2,[7] or ISO 648,[2] class A, fitted with an aspiration bulb.

**6.6 Burette,** capacity 25 ml and 50 ml, graduated in 0,1 ml divisions, ISO 385,[1] class A, autotitrator, ISO 8655-3,[8] as an alternative.

## 7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555.[5]

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

## 8 Preparation of the test sample and test portion

Prepare the sample in accordance with the method given in ISO 661.

According to the IV expected for the sample, weigh (6.3), to the nearest 0,001 g or 0,000 5 g, in a glass weighing scoop (6.1), the mass of test portion indicated in Table 1.

If the expected IV is not known, pre-test different test portions. The mass of the sample shall be such that there is an excess of Wijs reagent of between 50 % and 60 % of the amount added, i.e. 100 % to 150 % of the amount absorbed.

**Table 1 — Initial (theoretical) test portion mass for the expected iodine value**

| Expected iodine value | Initial mass for 150 % excess | Initial mass for 100 % excess | Initial mass accuracy | Solvent mixture |
|---|---|---|---|---|
| | g | g | g | ml |
| <3 | 10 | 10 | 0,001 | 25 |
| 3 | 8,461 | 10,576 | 0,001 | 25 |
| 5 | 5,077 | 6,346 | 0,001 | 25 |
| 10 | 2,538 | 3,173 | 0,001 | 20 |
| 20 | 0,846 | 1,586 | 0,001 | 20 |
| 40 | 0,634 | 0,793 | 0,001 | 20 |
| 60 | 0,432 | 0,529 | 0,001 | 20 |
| 80 | 0,317 | 0,397 | 0,001 | 20 |
| 100 | 0,254 | 0,317 | 0,000 5 | 20 |
| 120 | 0,212 | 0,264 | 0,000 5 | 20 |
| 140 | 0,181 | 0,227 | 0,000 5 | 20 |
| 160 | 0,159 | 0,198 | 0,000 5 | 20 |
| 180 | 0,141 | 0,176 | 0,000 5 | 20 |
| 200 | 0,127 | 0,159 | 0,000 5 | 20 |

## 9 Procedure

**9.1** Place the glass scoop containing the test portion in a 500 ml conical flask (6.2) and add the volume of solvent (5.5) indicated in Table 1. Add 25,00 ml of the Wijs reagent (5.6) by pipette (6.5). Insert the stopper, swirl the contents and place the flask in the dark.

Melt and dissolve fats and oils with a IV of 20 or less (hard or hardened fats) in warm solvent (60 °C). It is also recommended that all flasks and reagents be warmed before use. Closed vessels shall be used to avoid evaporation and change in concentration when warming the reagents.

NOTE The scoop remains in the flask.

**CAUTION — Do not use a mouth pipette for the Wijs reagent.**

**9.2** Prepare a blank with solvent and reagent as in 9.1 but omitting the test portion.

**9.3** For samples having an IV below 150, leave the flasks in the dark for 1 h. For samples with IVs above 150, and for polymerized products and oils containing conjugated fatty acids (such as tung oil, dehydrated castor oil) and any oils containing keto fatty acids (such as some grades of hydrogenated castor oil) and products oxidized to a considerable extent, leave the flasks in the dark for 2 h.

**9.4** At the end of the reaction time (9.3), add 20 ml of potassium iodide (5.2) and 150 ml of water (5.1). Titrate against standard sodium thiosulfate solution (5.4) until the yellow colour due to iodine has almost disappeared. Add a few drops of the starch solution (5.3) and continue the titration until the blue colour just disappears after vigorous shaking. Record the volume, $V_2$, of sodium thiosulfate solution required to reach the endpoint. Note that potentiometric determination of the endpoint is permissible.

**9.5** Carry out the determination using the blank solution (9.2) concurrently. In the blank determination, in 9.4, record the volume of sodium thiosulfate solution required to reach the endpoint as $V_1$.

## 10 Calculation

Calculate the iodine value (commonly known in the industry as IV), in grams per 100 g of fat, using the following formula.

$$w_I = \frac{12{,}69 \times c(V_1 - V_2)}{m}$$

where

$c$ is the concentration, in moles per litre, of the sodium thiosulfate solution (5.4);

$V_1$ is the volume, in millilitres, of sodium thiosulfate solution used for the blank test;

$V_2$ is the volume, in millilitres, of sodium thiosulfate solution used for the determination;

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

The results are rounded as indicated in Table 2.

**Table 2 — Rounding off of results**

| IV<br>g/100 g | Round to |
|---|---|
| ≤60 | 0,1 |
| >60 | 1 |

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. 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 limit, *r*

The repeatability limit, *r*, is the value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95 %.

Repeatability conditions are conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

### 11.3 Reproducibility limit, *R*

The reproducibility limit, *R*, is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions may be expected to be with a probability of 95 %.

Reproducibility conditions are conditions where independent test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment within short intervals of time.

## 12 Test report

The test report shall contain at least the following information:

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 (ISO 3961:2013);

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 test result(s);

e) the test result(s) obtained;

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

# Annex A
(informative)

# Interlaboratory test

The precision of the method has been established by an international interlaboratory test carried out in accordance with ISO 5725.[6] The test was organized by DIN in 2011.

The statistical results are given in Tables A.1 to A.3.

**Table A.1 — Statistical results for the Wijs method**

| Parameter | Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** |
| | Hardened vegetable oil | Coconut oil | Butter fat | Palm fat | Olive oil | Rapeseed oil | Sun-flower seed oil | Fish oil |
| Number of participating laboratories | 15 | 18 | 19 | 19 | 19 | 19 | 19 | 19 |
| Number of laboratories retained after eliminating outliers | 12 | 15 | 17 | 16 | 17 | 17 | 16 | 18 |
| Number of individual test in all laboratories | 24 | 30 | 34 | 32 | 34 | 34 | 32 | 36 |
| **Mean**, $\bar{w}_I$, g/100 g | **0,78** | **8,33** | **32,99** | **51,18** | **81,5** | **113,1** | **124,9** | **199,1** |
| Repeatability standard deviation, $s_r$, g/100 g | 0,07 | 0,07 | 0,17 | 0,21 | 0,6 | 0,8 | 0,6 | 1,1 |
| Coefficient of variation of repeatability, % | 9,1 | 0,9 | 0,5 | 0,4 | 0,7 | 0,7 | 0,5 | 0,6 |
| **Repeatability limit,** $r$ (2,8 $s_r$), g/100 g | **0,20** | **0,20** | **0,48** | **0,59** | **1,7** | **2,2** | **1,7** | **3,1** |
| Reproducibility standard deviation, $s_R$, g/100 g | 0,11 | 0,13 | 0,55 | 0,50 | 1,2 | 1,4 | 1,4 | 5,5 |
| Coefficient of variation of reproducibility, % | 14,6 | 1,6 | 1,7 | 1,0 | 1,5 | 1,2 | 1,1 | 2,7 |
| **Reproducibility limit,** $R$ (2,8 $s_R$), g/100 g | **0,32** | **0,36** | **1,54** | **1,40** | **3,4** | **3,9** | **3,9** | **15,3** |

**Table A.2 — Statistical results for the calculation from fatty acid composition (Annex B)**

| Parameter | Sample | | | | | | |
|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| | Hardened vegetable oil | Coconut oil | Butter fat | Palm fat | Olive oil | Rapeseed oil | Sun-flower seed oil |
| Number of participating laboratories | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| Number of laboratories retained after eliminating outliers | 17 | 15 | 16 | 16 | 18 | 14 | 15 |
| Number of individual test in all laboratories | 34 | 30 | 32 | 32 | 36 | 28 | 30 |
| **Mean**, $\bar{w}_I$, g/100 g | **0,22** | **8,61** | **30,16** | **51,49** | **80,3** | **111,3** | **124,5** |
| Repeatability standard deviation, $s_r$, g/100 g | 0,04 | 0,09 | 0,17 | 0,32 | 0,3 | 0,2 | 0,3 |
| Coefficient of variation of repeatability, % | 16,6 | 1,0 | 0,6 | 0,6 | 0,4 | 0,2 | 0,2 |
| **Repeatability limit,** $r$ (2,8 $s_r$), g/100 g | **0,10** | **0,25** | **0,48** | **0,91** | **0,8** | **0,5** | **0,8** |
| Reproducibility standard deviation, $s_R$, g/100 g | 0,23 | 0,87 | 1,85 | 1,00 | 1,6 | 0,6 | 0,7 |
| Coefficient of variation of reproducibility, % | 104,5 | 10,1 | 6,1 | 1,9 | 2,0 | 0,5 | 0,6 |
| **Reproducibility limit,** $R$ (2,8 $s_R$), g/100 g | **0,64** | **2,44** | **5,18** | **2,80** | **4,5** | **1,6** | **2,0** |

**Table A.3 — Comparison of $\bar{w}_I$, $r$, $R$ for both determination methods**

| Parameter | | Sample | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| | | Hardened vegetable oil | Coconut oil | Butter fat | Palm fat | Olive oil | Rapeseed oil | Sun-flower seed oil |
| **Mean,** $\bar{w}_I$, g/100 g | Wijs titration | **0,78** | **8,33** | **32,99** | **51,18** | **81,5** | **113,1** | **124,9** |
| | Calculation | **0,22** | **8,61** | **30,16** | **51,49** | **80,3** | **111,3** | **124,5** |
| | **Difference** | **0,56** | **0,28** | **2,83** | **0,31** | **1,20** | **1,78** | **0,40** |
| **Repeatability limit**, $r$, g/100 g | Wijs titration | **0,20** | **0,20** | **0,48** | **0,59** | **1,7** | **2,2** | **1,7** |
| | Calculation | **0,10** | **0,25** | **0,48** | **0,91** | **0,8** | **0,5** | **0,8** |
| **Reproducibility limit**, $R$, g/100 g | Wijs titration | **0,32** | **0,36** | **1,54** | **1,40** | **3,4** | **3,9** | **3,9** |
| | Calculation | **0,64** | **2,44** | **5,18** | **2,80** | **4,5** | **1,6** | **2,0** |

# Annex B
(informative)

# Calculated iodine value for non-fish oils

## B.1 General

This annex describes a method for calculating the IV of edible oils directly from fatty acid compositions determined by gas chromatography of methyl esters of fatty acids. It is applicable to triglycerides and free fatty acids and their hydrogenated products. For oils with an unsaponifiable content greater than 0,5 % mass fraction (e.g. fish oils), the calculation tends to produce underestimates, and hence is not applicable.

**IMPORTANT — While this procedure provides an IV, it is not intended to be a rapid method. The method gives two results from one analysis.**

## B.2 Procedure

**B.2.1** Determine the fatty acid composition of the oil or fatty acid mixture.

All positional isomers as well as all *cis*-/*trans*-isomers shall be included in the calculation.

**B.2.2** Calculate the IVs for groups of components as described in B.3.

NOTE The calculation tends to produce underestimates for materials with low IVs.

## B.3 Calculation

The IV for triglycerides is:

$$(w_{16:1}\times 0{,}950)+(w_{18:1}\times 0{,}860)+(w_{18:2}\times 1{,}732)+(w_{18:3}\times 2{,}616)+(w_{20:1}\times 0{,}785)+(w_{22:1}\times 0{,}723)$$

The IV for fatty acids is:

$$(w_{16:1}\times 0{,}997\,6)+(w_{18:1}\times 0{,}898\,6)+(w_{18:2}\times 1{,}810)+(w_{18:3}\times 2{,}735)+(w_{20:1}\times 0{,}817\,5)+(w_{22:1}\times 0{,}749\,7)$$

where

$w_{16:1}$ is the percentage mass fraction of hexadecenoic acid;

$w_{18:1}$ is the percentage mass fraction of octadecenoic acid;

$w_{18:2}$ is the percentage mass fraction of octadecadienoic acid;

$w_{18:3}$ is the percentage mass fraction of octadecatrienoic acid;

$w_{20:1}$ is the percentage mass fraction of eicosenoic acid;

$w_{22:1}$ is the percentage mass fraction of docosenoic acid.

The subscripts, in the format $n_C$:$n_{ene}$, denote the number of carbon atoms in the molecule, $n_C$, followed by the number of double bonds, $n_{ene}$.

Calculated IVs based on gas chromatographic (GC) fatty acid determination of non-triglyceride lipid materials, such as partial esters of glycerol, partial esters of sorbitol, sorbitan, and isosorbide mixtures, partial esters of polyoxyethylene sorbitol, sorbitan, and isosorbide mixtures or glycerol, provide the calculated IV of only the fatty acids used to prepare the partial esters. To obtain the actual IV of partial esters with non-fatty acid polyol diluents, the chlorinated Wijs reagent IV method should be used. IV values of partial esters via the Wijs method are lower than those obtained by GC because of the dilution effect of the polyol.

# Bibliography

[1] ISO 385, *Laboratory glassware — Burettes*

[2] ISO 648, *Laboratory glassware — Single-volume pipettes*

[3] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

[4] ISO 3696, *Water for analytical laboratory use — Specification and test methods*

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

[6] ISO 5725 (all parts), *Accuracy (trueness and precision) of measurement methods and results*

[7] ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

[8] ISO 8655-3, *Piston-operated volumetric apparatus — Part 3: Piston burettes*

[9] AOCS Official method Cd 1c-85, *Calculated iodine value*

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