INTERNATIONAL STANDARD

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Animal and vegetable fats and oils — Determination of ultraviolet absorbance expressed as specific UV extinction

Corps gras d'origines animale et végétale — Détermination de l'absorbance dans l'ultraviolet, exprimée sous la forme d'extinction spécifique en lumière ultraviolette

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 3656 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This third edition cancels and replaces the second edition (ISO 3656:1989), which has been technically revised.

Annex A of this International Standard is for information only.

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Animal and vegetable fats and oils — Determination of ultraviolet absorbance expressed as specific UV extinction

1   Scope

This International Standard specifies a method for the determination of the absorbance at ultraviolet wavelengths of animal and vegetable fats and oils.

2   Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 661:1989, *Animal and vegetable fats and oils — Preparation of test sample*

3   Principle

In a specified ultraviolet wavelength range, the absorbance of a sample in solution is measured spectrometrically. The absorbance at a concentration of 1 g per 100 ml in a 10 mm cell is calculated.

4   Reagent

Use only reagents of recognized analytical grade, unless otherwise stated.

4.1   Solvent: 2,2,4-trimethylpentane (isooctane), having an absorbance less than 0,12 at 230 nm and less than 0,05 at 250 nm against distilled water, measured in a cell of thickness 10 mm.

If 2,2,4-trimethylpentane is not available, cyclohexane or *n*-hexane having the characteristics specified above may be used instead.

5   Apparatus

The glassware used for the determination shall be thoroughly cleaned and rinsed with the solvent (4.1) before use so that it is free from impurities having an absorbance within the wavelength range of 220 nm to 320 nm.

Usual laboratory apparatus and, in particular, the following.

5.1   Spectrometer, preferably having a recording instrument, with quartz cells of thickness 10 mm, suitable for measurements at ultraviolet wavelengths.

Before use it is recommended that the wavelength and absorbance scales of the spectrometer be checked as follows.

a)   Wavelength scale

   This may be checked using a mercury lamp, in accordance with the instrument manufacturer's instructions. Alternatively a holmium glass plate, which displays sharp absorption peaks at 279,37 nm and 287,5 nm, may be used.

b) Absorbance scale

Prepare a 200 mg/l solution of analytical grade potassium chromate in 0,05 mol/l potassium hydroxide solution. Transfer 25 ml of this solution to a 500 ml volumetric flask and dilute to the mark with additional 0,05 mol/l potassium hydroxide solution. The absorbance of this solution, measured in a cell 10 mm thick at 275 nm against the 0,05 mol/l potassium hydroxide solution, should be $0,200 \pm 0,005$.

WARNING — Take care when handling potassium chromate which is a carcinogen by inhalation.

5.2   Volumetric flask, of 25 ml capacity.

6   Sampling

It is important that the laboratory receive a sample which is truly representative and has not 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.

7   Preparation of test sample

Prepare the test sample in accordance with ISO 661.

8   Procedure

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8.1   Test portion and preparation of the test solution

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Weigh into a 25 ml volumetric flask (5.2), to the nearest 0,1 mg, an amount of the test sample (clause 7), generally 0,05 g to 0,25 g, necessary to obtain absorbance values between 0,2 and 0,8.

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Dissolve the test portion in a few millilitres of the solvent (4.1) at ambient temperature and then make up to the mark with the same solvent. Mix thoroughly.

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If the concentration of test sample in the test solution is greater than 1 g per 100 ml of solution, this shall be stated in the test report.

8.2   Determination

Rinse a quartz cell (5.1) three times with the test solution (8.1). Fill the cell with the test solution and measure the absorbance against the solvent used for dilution, by means of the spectrometer (5.1) over a wavelength range from 220 nm to 320 nm, either continuously or at intervals of 1 nm or 2 nm, reducing the intervals to 0,5 nm in the regions of maximum and minimum absorbance.

NOTE   It may not be necessary to measure the absorbance over the full wavelength range.

If the absorbance value obtained exceeds 0,8, dilute the test solution as appropriate and repeat the determination.

9   Expression of results

The absorbance of a solution of fat or oil at a concentration of 1 g per 100 ml (1 %), measured using an optical path length of 10 mm and at a wavelength λ, is given by the following formula:

$$E_{1cm(\lambda)}^{1\%} = \frac{A_{(\lambda)}}{w}$$

where

$A_{(\lambda)}$ is the absorbance at wavelength λ;

w is the concentration, in grams per 100 ml, of the test sample in the test solution.

NOTE λ is usually 232 nm and 268 nm.

10 Precision

10.1 Interlaboratory test

Details of an inlaboratory test are summarized in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges other than those given.

10.2 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 the overall repeatability limit (r): 0,026 at 232 nm and 0,085 at 268 nm.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the overall reproducibility limit (R): 0,396 at 232 nm and 0,11 at 268 nm.

11 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 test result;

⎯ the test result obtained or, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was carried out in 1998 in accordance with ISO 5725[1] by the Istituto Sperimentale per la Elaiotecnica, Pescara, Italy.

Sixteen laboratories from eight countries participated. The results are summarized in Tables A.1 and A.2.

Table A.1 — UV extinction at 232 nm

	Sample				
	Sunflowerseed oil	**EV olive oil**	**Rapeseed oil**	**Palm kernel oil**	**Soyabean oil**
Number of laboratories after eliminating outliers	16	16	16	16	16
Number of accepted results	13	14	13	13	14
Mean value	2,515	2,220	3,459	2,221	3,665
Repeatability standard deviation (s_r)	0,119 85	0,063 94	0,058 2	0,037 4	0,109 31
Repeatability limit (r)	0,338 94	0,180 82	0,164 59	0,105 77	0,309 13
Reproducibility standard deviation (s_R)	0,186 58	0,083 85	0,140 5	0,098 3	0,180 84
Reproducibility limit (R)	0,527 65	0,237 13	0,397 33	0,277 99	0,511 42

At 232 nm:

— overall repeatability standard deviation s_r = 0,08

— overall repeatability limit r = 0,226

— overall reproducibility standard deviation s_R = 0,14

— overall reproducibility limit R = 0,396

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1) ISO 5725:1986 (now withdrawn) was used to evaluate the precision data.

Table A.2 — UV extinction at 268 nm

	Sample				
	Sunflowerseed oil	**EV olive oil**	**Rapeseed oil**	**Palm kernel oil**	**Soyabean oil**
Number of laboratories after eliminating outliers	16	16	16	16	16
Number of acceptable results	13	12	11	12	13
Mean value	1,872	0,157	0,627	0,675	1,437
Repeatability standard deviation (s_r)	0,046 98	0,021 5	0,022 05	0,021 1	0,032 92
Repeatability limit (r)	0,132 86	0,060 8	0,062 36	0,059 67	0,093 10
Reproducibility standard deviation (s_R)	0,062 63	0,020 21	0,037 9	0,029 26	0,051 65
Reproducibility limit (R)	0,177 12	0,057 15	0,107 18	0,082 75	0,146 07

At 268 nm:

— overall repeatability standard deviation s_r = 0,03

— overall repeatability limit r = 0,085

— overall reproducibility standard deviation s_R = 0,04

— overall reproducibility limit R = 0,11