

# INTERNATIONAL STANDARD

**ISO**  
**3656**

Second edition  
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## **Animal and vegetable fats and oils — Determination of ultraviolet absorbance**

*Corps gras d'origines animale et végétale — Détermination de l'absorbance dans  
l'ultraviolet*

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Reference number  
ISO 3656 : 1989 (E)

## 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 approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 3656 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

This second edition cancels and replaces the first edition (ISO 3656 : 1977), of which it constitutes a technical revision.

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## Introduction

Conjugated dienes show a wide absorption band in the ultraviolet region at about 232 nm. Conjugated trienes show a triple absorption band in the neighbourhood of 268 nm. Oxidation products of unsaturated fatty acids, if they have a conjugated diene structure (for example, linoleic hydroperoxide), absorb at about 232 nm. Secondary oxidation products absorb at about 268 nm.

Therefore, the determination of absorbance at about 232 nm or at about 268 nm permits the detection and evaluation of conjugated oxidation products and, in some cases, a determination of the conjugated polyenic fatty acids content.

It should be noted that dark-coloured oils (e.g. dark palm oils) having a high carotene content will exhibit absorption at 268 nm to 270 nm.

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# Animal and vegetable fats and oils — Determination of ultraviolet absorbance

## 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 references

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

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

ISO 5555 : 1983, *Animal and vegetable fats and oils — Sampling*.

## 3 Principle

Spectrometric measurement, in a specified ultraviolet wavelength range, of the absorbance of a sample in solution. Calculation of the absorbance at a concentration of 1 g per 100 ml.

## 4 Reagent

**Solvent:** 2,2,4-trimethylpentane (iso-octane), 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.

NOTE — If 2,2,4-trimethylpentane is not available, *n*-hexane or cyclohexane, 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 (clause 4) before use so

that it is free from impurities having an absorbance within the wavelength range 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.

NOTE — 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 the 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  $0,200 \pm 0,005$ .

**5.2 Volumetric flask**, of 25 ml capacity.

## 6 Sampling

Sampling shall have been carried out in accordance with ISO 5555.

## 7 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

## 8 Procedure

### 8.1 Test portion and preparation of the test solution

Weigh, to the nearest 0,1 mg, into a 25 ml volumetric flask (5.2), 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.

Dissolve the test portion in a few millilitres of the solvent (clause 4) at ambient temperature and then make up to the mark with the same solvent. Mix thoroughly.

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.

### NOTES

- 1 It may not be necessary to measure the absorbance over the full wavelength range.
- 2 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 1 cm and at a wavelength  $\lambda$ , is given by the following formula :

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

where

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

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

NOTE:  $\lambda$  is usually approximately 232 nm and 268 nm.

## 10 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, together with any circumstances that may have influenced the result.

The test report shall include all details required for complete identification of the sample.