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# INTERNATIONAL STANDARD 3656

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## Animal oils and fats – Determination of specific extinction in ultra-violet light

*Corps gras d'origine animale – Détermination de l'absorbance spécifique en rayonnement ultra-violet*

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## FOREWORD

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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.

International Standard ISO 3656 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in January 1975.

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It has been approved by the member bodies of the following countries :

Australia	Germany	Poland
Austria	Ghana	Portugal
Belgium	India	Romania
Brazil	Iran	South Africa, Rep. of
Bulgaria	Ireland	Spain
Canada	Israel	Thailand
Egypt, Arab Rep. of	Mexico	Turkey
Ethiopia	Netherlands	United Kingdom
France	New Zealand	Yugoslavia

No member body expressed disapproval of the document.

# Animal oils and fats – Determination of specific extinction in ultra-violet light

## 0 INTRODUCTION

The autoxidation products of oils and fats display characteristic spectra in the ultra-violet region : linoleic hydroperoxide and the conjugated dienes which may result from its decomposition show an absorption band at about 232 nm; secondary products of autoxidation, and particularly ethylenic diketones, show an absorption band at about 268 nm.

Conjugated trienes, which may exist naturally in certain fats (for example, premier jus tallows) or which are formed during industrial treatment (for example, on bleaching by bleaching earths) show a triple absorption band of which the principal peak is in the neighbourhood of 268 nm, a secondary peak at about 278 nm, and minima adjacent to the principal peak at about 262 and 274 nm.

Therefore, a determination of the absorbance  $E_{1\text{ cm}}^{1\%}$  at 232 nm can afford an indication of the state of autoxidation of a fat.

Also, the determination of the absorbance  $E_{1\text{ cm}}^{1\%}$  at 268 nm and of variations between 262 and 274 nm, or, perhaps, between 277 and 283 nm, can reveal the presence of secondary products of autoxidation and of conjugated trienes in fats.

## 1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for the determination of the specific extinction in ultra-violet light of animal oils and fats intended for human and animal consumption.

NOTE – The method may be used to demonstrate the presence or absence of refined oils and fats in unrefined oils and fats, such as lard, that do not themselves contain (or that contain only negligible amounts of) conjugated trienes, by comparing the values of  $E$ ,  $T$  and  $Q$  (see clause 8) obtained for the product under examination with those for the unrefined and unadulterated product.

## 2 REFERENCE

ISO . . . , *Animal and vegetable oils and fats – Sampling*.<sup>1)</sup>

## 3 PRINCIPLE

Spectrophotometric measurement, in a specified wavelength range in the ultra-violet, of the absorbance of a homogenized and diluted sample, the absorbance depending on the conjugated trienes content among other factors.

## 4 REAGENT

4.1 Hexane, or cyclohexane or trimethylpentane (isooctane), optically pure from 220 to 282 nm.

## 5 APPARATUS

5.1 Spectrophotometer with quartz prism (or gratings) and quartz cells, suitable for measurements with ultra-violet light.

5.2 One-mark volumetric flask, 25 ml capacity, complying with ISO 1042.

5.3 Water bath capable of being controlled at a temperature of 50 to 60 °C.

5.4 Analytical balance.

## 6 SAMPLING

See ISO . . .

## 7 PROCEDURE

NOTE – The glassware used for the determination shall be thoroughly cleaned before use. Make sure that it is free of impurities having an absorbance within the range of 260 to 275 nm by testing it with one of the solvents (4.1).

### 7.1 Preparation of test sample

Melt the sample, if necessary, at about 10 °C above its melting point and filter at this temperature through a dry, fast filter paper to which a mixture of 4 g of anhydrous sodium sulphate and 1 g of filter aid has been added. Repeat as necessary until the filtrate is perfectly clear.

1) In preparation.

**7.2 Test portion**

Weigh, to the nearest 0,000 1 g, into the volumetric flask (5.2), an amount of the test sample (7.1) sufficient to give absorbance values within the range between 0,2 and 0,6 (usually about 0,2 g).

**7.3 Determination**

Dissolve the test portion in a few millilitres of one of the solvents (4.1) and dilute to the mark with the same solvent at 20 °C.

Transfer the contents of the flask to a quartz cell and measure the absorbance of the fat solution, against the solvent used, by means of the spectrophotometer (5.1) over a wavelength range from 262 to 274 nm, either continuously or at intervals of 1 (or 2) nm, reducing the intervals to 0,5 nm in regions of maximum and minimum absorbances.

NOTE — An extension of the wavelength range beyond these limits may be made.

**8 EXPRESSION OF RESULTS**

Calculate the results as follows :

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

$$T = 100 \left[ E_{1\text{ cm}}^{1\%}(\lambda) - \frac{E_{1\text{ cm}}^{1\%}(\lambda - 4\text{ nm}) + E_{1\text{ cm}}^{1\%}(\lambda + 4\text{ nm})}{2} \right]$$

$$Q = \frac{T}{E_{1\text{ cm}}^{1\%}(\lambda)}$$

where

$E_{1\text{ cm}}^{1\%}$  is the specific extinction of a solution with a concentration of 1 % using a standard path length of 1 cm;

$\lambda$  is the wavelength, in nanometres, corresponding to the absorbance maximum near 268 nm;

$D(\lambda)$  is the maximum absorbance occurring at a wavelength near 268 nm;

$c$  is the concentration, in grams per 100 ml, of the solution;

$d$  is the thickness, in centimetres, of the solution layer;

$T$  is the triene value;

$Q$  is the quotient value.

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**9 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 (in particular, the solvent used), as well as any circumstances that may have influenced the result.

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