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Animal and vegetable fats and oils -- Determination of anisidine value

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Corps gras d'origines animale et végétale -- Détermination de l'indice d'anisidine

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# INTERNATIONAL STANDARD

**ISO  
6885**

Second edition  
1998-07-01

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## **Animal and vegetable fats and oils — Determination of anisidine value**

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

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Reference number  
ISO 6885:1998(E)

**ISO 6885:1998(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 voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 6885 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition of ISO 6885 cancels and replaces the first edition (ISO 6885:1988), of which it constitutes a minor revision.

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

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

## 1 Scope

This International Standard specifies a method for the determination of the anisidine value, which is a measure of the amount of aldehydes (principally 2-alkanals), in 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 listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

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ISO 661:1989, *Animal and vegetable fats and oils — Preparation of test sample*.  
191953aa600a/sist-iso-6885-1998

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

#### anisidine value

one hundred times the increase in absorbance, measured at a wavelength of 350 nm in a 10 mm cell, of a test solution when reacted with *p*-anisidine under the test conditions specified in this International Standard

NOTE In practice the anisidine value is calculated on the basis of 1 g of test sample in 100 ml of solution.

## 4 Principle

Preparation of a test solution in 2,2,4-trimethylpentane (iso-octane). Reaction with an acetic acid solution of *p*-anisidine and measurement of the increase in absorbance at 350 nm. Calculation of the anisidine value.

## 5 Reagents

Use only reagents of recognized analytical grade, and water complying with grade 3 of ISO 3696.

**5.1 Sodium sulfate** ( $\text{Na}_2\text{SO}_4$ ), anhydrous.

**5.2 2,2,4-Trimethylpentane (iso-octane)**, having zero absorbance in the wavelength range 300 nm to 380 nm.

**5.3 4-Methoxyaniline (*p*-anisidine)**, anhydrous cream-coloured crystals.

**WARNING — *p*-Anisidine is toxic and care should be taken to avoid contact with the skin.**

Store the *p*-anisidine in a dark bottle at 0 °C to 4 °C in the dark.

No discoloration (grey or pink) shall be observed. If this is present, purify the *p*-anisidine as follows.

Dissolve 4 g of *p*-anisidine in 100 ml of water at 75 °C. Add 0,5 g of sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) and 2 g of charcoal. Stir for 5 min and filter through a medium retention filter paper to give a clear solution. Cool the filtrate to 0 °C and leave at this temperature for not less than 4 h. Filter off the crystals, preferably under vacuum, and wash with a small volume of water at about 0 °C. Dry in a vacuum desiccator containing an efficient desiccant.

**5.4 Glacial acetic acid**, of water content not greater than 0,1 % (*m/m*).

### 5.5 Anisidine reagent

On the day of use prepare the minimum quantity of reagent required for the analysis, in view of its toxicity and limited life. Prepare, for example, 50 ml of reagent as follows.

Dissolve 0,125 g of the *p*-anisidine (5.3) in the glacial acetic acid (5.4) in a 50 ml volumetric flask and dilute to the mark with the same solvent, avoiding exposure to strong light.

Check the absorbance before use and discard the reagent if this rises above 0,2. In any case, discard any reagent left over on the day of use.

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## 6 Apparatus

Usual laboratory apparatus and, in particular, the following.

**6.1 Spectrometer**, double- or single-beam, suitable for use at a wavelength of 350 nm, with cells of optical path length 10 mm.

When a double-beam spectrometer is used, it is recommended that a pair of matched 10 mm cells be used.

**6.2 Volumetric flasks**, of 25 ml capacity.

**6.3 Test tubes**, of 10 ml capacity, fitted with ground glass stoppers.

**6.4 Pipettes**, of 1 ml and 5 ml capacities, equipped with a safety suction device.

## 7 Sampling

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

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

## 8 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

If the moisture content of the sample is greater than 0,10 % ( $m/m$ ), it should be dried using the following procedure.

Add sodium sulfate (5.1) in the proportion of 1 g to 2 g per 10 g of the thoroughly mixed sample, at a temperature of not more than 10 °C above the melting point in the case of a solid fat. Stir thoroughly and filter, maintaining the temperature to prevent solidification.

Take care to exclude extraneous moisture during the procedure because it can affect the equilibrium reaction during which water is produced.

## 9 Procedure

NOTE If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.1 to 9.5.

### 9.1 Test portion and preparation of test solution

Weigh, to the nearest 1 mg, a sufficient mass of the prepared test sample (clause 8) directly into a 25 ml volumetric flask. Preheat solid samples to 10 °C above their melting point. Dissolve the sample in 5 ml to 10 ml of the 2,2,4-trimethylpentane (5.2) and make up to the mark with the same solvent.

NOTE The size of the test portion depends on the quality of the sample and the characteristics of the spectrometer used, and should be chosen to avoid readings near the upper and lower ends of the scale. In general 0,4 g to 4,0 g is used.

### 9.2 Unreacted test solution

By means of a pipette (6.4), transfer 5 ml of the test solution (9.1) to a test tube (6.3). Add 1 ml of glacial acetic acid (5.4), stopper the tube and shake well.

### 9.3 Colour development

Transfer, by means of a pipette (6.4) 5 ml of the test solution (9.1) to a test tube (6.3). For a blank test, transfer 5 ml of 2,2,4-trimethylpentane (5.2) to another test tube (6.3). Add to each of the test tubes, by means of a pipette, 1 ml of the anisidine reagent (5.5). Stopper both tubes and shake well. Keep the test tubes in the dark at 23 °C ± 1 °C for 8 min.

Within a further 2 min, transfer each of the solutions to a clean, dry spectrometer cell. After a total reaction time of 10 min ± 1 min from the addition of the anisidine reagent, follow the procedure specified in 9.4.

### 9.4 Spectrometric measurement

Adjust the spectrometer with 2,2,4-trimethylpentane (5.2).

Measure the absorbance of the reacted solution (9.3), of the unreacted test solution (9.2), and of the blank (9.3), respectively, against 2,2,4-trimethylpentane (5.2).

### 9.5 Absorbance range

If the measured absorbance of the reacted solution is not in the range 0,2 to 0,8, repeat the determination (9.2 to 9.4) with an adjusted amount of test sample.

If the measured absorbance of the blank exceeds 0,2, purify the anisidine reagent as described in 5.3, and prepare fresh anisidine reagent (5.5).

## 10 Expression of results

The anisidine value (AV) of the sample is equal to

$$AV = \frac{100QV}{m} [1,2(A_1 - A_2) - A_0]$$

where

$V$  is the volume in which the test sample is dissolved, in millilitres ( $V = 25$  ml);

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

$Q$  is the sample content of the measured solution based on which the anisidine value is expressed, in grams per millilitre ( $Q = 0,01$  g/ml);

$A_0$  is the absorbance of the unreacted test solution (9.2)

$A_1$  is the absorbance of the reacted solution (9.3);

$A_2$  is the absorbance of the blank (9.3);

Report the results to 1 decimal place.

## 11 Precision

### 11.1 Results of interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in annex A. The values derived from this interlaboratory test may not be applicable to 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 exceed 0,3 for an anisidine value of about 2.

### 11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in two different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed 2,0 for an anisidine value of about 2.

## 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, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results;
- the test results obtained;
- if the repeatability has been checked, the final quoted result obtained.



## Annex A (informative)

### Results of interlaboratory test

An interlaboratory test carried out at the International level in 1988 by FOSFA International, in which 23 laboratories participated, each performing two determinations on each sample, gave the statistical results (determined in accordance with ISO 5725<sup>1)</sup>) given in table A.1.

Table A.1

| Sample   | Crude colza<br>oil A | Crude colza<br>oil B | Refined palm<br>oil C | Refined palm<br>oil D |
|--|----------------------|----------------------|-----------------------|-----------------------|
| Number of laboratories retained after eliminating outliers | 20                   | 20                   | 20                    | 20                    |
| Mean   | 2,0                  | 2,0                  | 2,3                   | 2,3                   |
| Standard deviation of repeatability, $s_r$                 | 0,08                 | 0,12                 | 0,11                  | 0,10                  |
| Coefficient of variation of repeatability, %               | 4,1                  | 5,8                  | 4,8                   | 4,6                   |
| Repeatability limit, $r$ ( $2,83 \times s_r$ )             | 0,2                  | 0,3                  | 0,3                   | 0,3                   |
| Standard deviation of reproducibility, $s_R$               | 0,71                 | 0,73                 | 0,70                  | 0,69                  |
| Coefficient of variation of reproducibility, %             | 35                   | 37                   | 30                    | 31                    |
| Reproducibility limit, $R$ ( $2,83 \times s_R$ )           | 2,0                  | 2,1                  | 2,0                   | 2,0                   |

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<sup>1)</sup> ISO 5725:1986 (now withdrawn) was used to obtain the precision data.