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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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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents	Page
Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents	1
6 Apparatus	2
7 Sampling	2
8 Preparation of test sample	2
9 Procedure	3
9.1 Test portion and preparation of test solution	3
9.2 Unreacted test solution	3
9.3 Reacted test solution	3
9.4 Blank	3
9.5 Spectrometric measurement	3
9.6 Absorbance range	3
10 Expression of results	4
11 Precision	4
11.1 Interlaboratory tests	4
11.2 Repeatability	4
11.3 Reproducibility	4
12 Test report	5
Annex A (informative) Results of the interlaboratory test	6
Bibliography	7

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This fourth edition cancels and replaces the third edition (ISO 6885:2006), which has been technically revised by adding a sentence to the Scope and deleting a column in [Table A.1](#).

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 in animal and vegetable fats and oils. This is a measure of the amount of aldehydes present (principally α , β -unsaturated aldehydes).

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this International Standard.

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*

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

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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 1 to entry: The anisidine value has no dimensions, and is calculated and quoted on the basis of 1 g of the test sample in 100 ml of a mixture of solvent and reagent.

4 Principle

A test solution is prepared in isooctane (2,2,4-trimethylpentane). It is reacted with an acetic acid solution of *p*-anisidine. The increase in absorbance at 350 nm is measured. The anisidine value is calculated.

5 Reagents

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

5.1 Sodium sulfate (Na_2SO_4), anhydrous.

5.2 Isooctane (2,2,4-trimethylpentane), having an absorbance not exceeding 0,01 against water 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 shall 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 coloration (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 (Na₂SO₃) 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 at least 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 % (mass fraction).

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 against isooctane before use and discard the reagent when the difference is larger than 0,2. In any case, discard any reagent left over on the day of use.

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

A representative sample should have been sent to the laboratory. It should not have 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.

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 % (mass fraction), 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

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 isooctane (5.2) and make up to the mark with the same solvent.

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. Keep the test tube in the dark at (23 ± 3) °C for 8 min.

Within a further 2 min, transfer the solutions to a clean, dry spectrometer cell. After a total reaction time of $10 \text{ min} \pm 1 \text{ min}$, follow the procedure specified in 9.5.

9.3 Reacted test solution

Transfer, by means of a pipette (6.4), 5 ml of the test solution (9.1) to a test tube (6.3). Add, by means of a pipette (6.4), 1 ml of the anisidine reagent (5.5). Stopper the tube and shake well. Keep the test tube in the dark at (23 ± 3) °C for 8 min.

Within a further 2 min, transfer the solutions to a clean, dry spectrometer cell. After a total reaction time of $10 \text{ min} \pm 1 \text{ min}$ from the addition of the anisidine reagent, follow the procedure specified in 9.5.

9.4 Blank

Transfer, by means of a pipette (6.4), 5 ml of isooctane (5.2) to a test tube (6.3). Add, by means of a pipette (6.4), 1 ml of the anisidine reagent (5.5). Stopper the tube and shake well. Keep the test tube in the dark at (23 ± 3) °C for 8 min.

Within a further 2 min, transfer the solutions to a clean, dry spectrometer cell. After a total reaction time of $10 \text{ min} \pm 1 \text{ min}$ from the addition of the anisidine reagent, follow the procedure specified in 9.5.

9.5 Spectrometric measurement

Adjust the zero absorption of the spectrometer with isooctane (5.2) at 350 nm.

Measure the following absorbances against isooctane (5.2):

- A_1 of the reacted solution (9.3),
- A_0 of the unreacted test solution (9.2), and
- A_2 of the blank (9.4).

9.6 Absorbance range

If the measured absorbance A_1 of the reacted solution (9.3) 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 A_2 of the blank exceeds 0,2, purify the anisidine reagent as described in [5.3](#), and prepare fresh anisidine reagent ([5.5](#)). Repeat this test with the fresh anisidine reagent.

10 Expression of results

10.1 The anisidine value (AV) of the sample is equal to Formula (1):

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

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 ([9.4](#));

1,2 is the correction factor for the dilution of the test solution with 1 ml of the reagent or glacial acetic acid.

Report the results to 1 decimal place.

10.2 When assessing the oxidative deterioration of an oil, the total oxidation value, or 'totox value' (TV), may be helpful. The calculation is shown in Formula (2) [with the peroxide value (PV) expressed in meq O₂/kg]:

$$TV = (2 \times PV) + AV \quad (2)$$

11 Precision

11.1 Interlaboratory tests

Details of two interlaboratory tests on the precision of the method are summarized in [Annex A](#). The values derived from these interlaboratory tests 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 the value of r given in [Table 1](#).

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 the value of R given in [Table 1](#).

Table 1 — Repeatability limit (*r*) and reproducibility limit (*R*)

Anisidine value	Range of variation	<i>r</i>	<i>R</i>
AV (mean of two determinations)	0 to 100	$0,034 AV + 0,31$	$0,19 AV + 1,41$

12 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method, with reference to this International Standard, i.e. ISO 6885;
- 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 results;
- e) the test results obtained;
- f) if the repeatability has been checked, the final quoted result obtained.

Annex A (informative)

Results of the interlaboratory test

An interlaboratory test carried out at international level in 2004 by ITERG (France), in which 18 laboratories participated (9 countries: Argentina, Canada, France, Germany, Hungary, Netherlands, Portugal, UK, USA), each performing 2 determinations on each sample, gave the statistical results (determined in accordance with ISO 5725-2) given in [Table A.1](#).

Table A.1 — Results of the interlaboratory test

Sample	Soya oil	Crude canola oil	Walnut oil	Fish oil	Fish oil	Poultry fat	Used frying oil
Number of laboratories retained after eliminating outliers	16	17	17	16	17	15	16
Mean	3,46	0,95	6,86	25,46	31,54	4,59	96,80
Standard deviation of repeatability, s_r	0,09	0,08	0,17	0,31	0,72	0,28	1,22
Coefficient of variation of repeatability, %	2,6	8,2	2,5	1,2	2,3	6,0	1,3
Repeatability limit, r ($2,8 \times s_r$)	0,25	0,22	0,48	0,86	2,02	0,78	3,43
Standard deviation of reproducibility, s_R	0,27	0,39	0,52	1,75	3,79	1,80	6,74
Coefficient of variation of reproducibility, %	7,9	41,4	7,6	6,9	12,0	39,3	7,0
Reproducibility limit, R ($2,8 \times s_R$)	0,77	1,10	1,46	4,89	10,61	5,05	18,86

Bibliography

- [1] ISO 5555, *Animal and vegetable fats and oils — Sampling*
- [2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [4] IUPAC 2.504, *Determination of the p-anisidine value (p-A.V.)*

