INTERNATIONAL STANDARD

ISO
6885
First edition
1988-07-01

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION
ORGANISATION INTERNATIONALE DE NORMALISATION
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Animal and vegetable fats and oils — Determination of anisidine value

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

Reference number
ISO 6885 : 1988 (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 6885 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

Printed in Switzerland

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-alkenals), 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.

ISO 661 : 1980, *Animal and vegetable fats and oils — Preparation of test sample.*

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

3 Definition

For the purposes of this International Standard, the following definition applies.

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 conditions of the test.

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

All the reagents shall be of recognized analytical grade and the water used shall be distilled water or water of equivalent purity.

5.1 Sodium sulfate (Na_2SO_4), anhydrous.

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

5.3 Anisidine reagent.

5.3.1 4-methoxy aniline (*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; otherwise 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 (Na_2SO_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.

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

5.3.3 Preparation

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 (5.3.1) in the glacial acetic acid (5.3.2) in a 50 ml volumetric flask and dilute to the mark with the same solvent, avoiding exposure to strong light. If possible, prepare the reagent on the day of use. Otherwise, store in a dark bottle at 0 °C to 4 °C in the dark.

Check the absorbance before use and discard the reagent if it rises above 0,2. In any case, discard any reagent 3 days after preparation.

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 thickness 10 mm.

NOTE — 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

See ISO 5555.

8 Preparation of the test sample

See 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.

NOTE — Care is essential 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 milligram, a sufficient mass of test sample (clause 8) (solid samples shall be preheated to 10 °C above the melting point) directly into a 25 ml volumetric flask. Dissolve it 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 Blank test

Carry out a blank test in parallel with the determination, by the same procedure, using the same quantities of all reagents as in the determination but using 5 ml of the 2,2,4-trimethylpentane instead of the test solution.

9.3 Determination

9.3.1 Colour development

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, 1 ml of the anisidine reagent (5.3) to the test-tube, stopper it and shake well. Keep the test-tube in the dark at 23 °C ± 1 °C for about 8 min.

Transfer the solution 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 either 9.3.2 or 9.3.3.

9.3.2 Double-beam spectrometer

Measure the absorbance at 350 nm of the reacted test solution (9.3.1) against the blank test (9.2).

Empty, clean and dry the cell from the reacted test solution and use it to measure the absorbance of the unreacted test solution (9.1) against the blank test.

9.3.3 Single-beam spectrometer

Measure the absorbances at 350 nm of the reacted test solution (9.3.1), of the blank test (9.2) and of the unreacted test solution (9.1) against the 2,2,4-trimethylpentane (5.2).

Alternatively, measure the absorbances of the reacted and unreacted test solutions against the blank test (9.2).

9.3.4 Absorbance range

If the measured absorbance is not in the range 0,2 to 0,8, repeat the determination (9.3.2 or 9.3.3) with an adjusted amount of sample.

9.4 Number of determinations

Carry out two determinations on separate test portions from the same test sample.

10 Expression of results

10.1 Method of calculation

10.1.1 For a double-beam spectrometer and for the alternative measurement for a single-beam spectrometer, the anisidine value of the sample is equal to

$$\frac{25\,(1{,}2\,A_1 - A_0)}{m}$$

where

A_0 is the absorbance of the unreacted test solution measured against the blank test;

A_1 is the absorbance of the reacted test solution measured against the blank test;

m is the mass, in grams, of the test portion.

10.1.2 For the former measurement for a single-beam spectrometer, the anisidine value of the sample is equal to

$$\frac{25\,[1{,}2\,(A_1 - A_2) - A_0]}{m}$$

where

A_0 is the absorbance of the unreacted test solution measured against the 2,2,4-trimethylpentane;

A_1 is the absorbance of the reacted test solution measured against the 2,2,4-trimethylpentane;

A_2 is the absorbance of the blank test measured against the 2,2,4-trimethylpentane;

m is the mass, in grams, of the test portion.

10.1.3 Report the anisidine value as the arithmetic mean of the two determinations (9.4) provided that the repeatability requirement (10.2.2) is met.

Give the result to one decimal place.

10.2 Precision

10.2.1 Results of interlaboratory tests

An interlaboratory test carried out at the international level in 1986 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[1]) given in table 1.

10.2.2 Repeatability

By application of the above results (10.2.1) for an anisidine value of about 2, the difference between the values of two determinations, carried out in rapid succession (or simultaneously) by the same operator using the same apparatus on the same test sample, shall not exceed 0,3 (absolute).

10.2.3 Reproducibility

By application of the above results (10.2.1) for an anisidine value of about 2, the difference between the final result values obtained by two laboratories using this method for the analysis of the same laboratory sample, shall not exceed 2,0 (absolute).

11 Test report

The test report shall specify the method used and the results obtained. It shall also mention 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 report shall include all information necessary for the complete identification of the sample.

Table 1

Sample	Crude colza oil A	Crude colza oil B	Refined palm oil C	Refined palm oil D
Number of laboratoires 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, 2,83 × 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, 2,83 × s_R	2,0	2,1	2,0	2,0

1) ISO 5725 : 1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

UDC [664.3 + 665.2/.3] : 543.42 : 547.28

Descriptors : agricultural products, animal fats, vegetable fats, animal oils, vegetable oils, chemical analysis, determination, absorbance.

Price based on 3 pages