INTERNATIONAL STANDARD

ISO 12193

First edition
1994-11-01

Animal and vegetable fats and oils — Determination of lead content — Graphite furnace atomic absorption method

Corps gras d'origines animale et végétale — Détermination de la teneur en plomb — Méthode par spectrométrie d'absorption atomique avec four en graphite.

Reference number
ISO 12193:1994(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 12193 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Annex A of this International Standard is for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland
Printed in Switzerland

Animal and vegetable fats and oils — Determination of lead content — Graphite furnace atomic absorption method

1 Scope

This International Standard specifies a method for the determination of trace amounts (< 0,1 mg/kg) of lead in animal and vegetable fats and oils, referred to hereinafter as fats.

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 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

3 Principle

Vaporization of a test portion of the fat in a graphite furnace connected to an atomic absorption spectrometer, previously calibrated using standard solutions of an organo compound of lead. Calculation of the lead content from the absorbance at a selected wavelength of 283,3 nm.

4 Reagents

Use only reagents of recognized analytical grade and water in accordance with grade 2 of ISO 3696.

4.1 Cyclohexane

4.2 Dilution oil, for example a refined edible oil, liquid at ambient temperature.

Store the oil in a metal-free poylethylene or polypropylene bottle (5.1). The lead content of the oil shall be not greater than 1 µg/kg.

NOTE 1 Refined edible oils have a lead content below 1 µg/kg.

4.3 Standard stock solution

By appropriate dilution of an organo-lead standard with the dilution oil (4.2), prepare a stock solution with a lead content of 10 mg/kg.

NOTE 2 A suitable standard is available from Continental Oil Company, Ponca City, Oklahoma, USA (Conostan, 5 000 mg/kg), or Merck, D-1600 Darmstadt, Germany (1 000 mg/kg).[1]

4.4 Standard working solutions

Prepare working solutions as required on the day of use with lead contents of 0,020 mg/kg, 0,050 mg/kg and 0,100 mg/kg, by diluting the stock solution (4.3) with the dilution oil (4.2).

4.5 *n*-Heptane

---

[1] This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4.6 Matrix modifier

Dissolve 2 g of soya lecithin with a phosphorus content of approximately 2 % (*m/m*) in 100 ml of cyclohexane.

NOTE 3 A suitable lecithin is available from Unimills Zwijndrecht, Netherlands, or Verein Deutscher Oelfabriken, D-6800 Mannheim, Germany (Bolec M), or Lucas Meyer GmbH & Co., D-2000 Hamburg, Germany.[1]

4.7 Argon, minimum purity 99,99 %.

NOTE 4 If argon is not available, nitrogen may be used as the purge gas. At temperatures above 2 300 °C, nitrogen forms the toxic gas hydrogen cyanide, therefore continuous ventilation of the furnace area is necessary.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Bottles and **caps**, made of polyethylene or polypropylene, metal-free, of 20 ml capacity.

NOTE 5 If necessary, the polyethylene or polypropylene bottles and caps may be rendered metal-free by cleaning thoroughly with warm nitric acid soluton [$c(\text{HNO}_3) = 1$ mol/l], rinsing with water and then drying in an oven at about 80 °C.

5.2 Micropipettes, of 20 µl capacity.

5.3 Pipette tips

5.4 Electric oven, capable of being maintained at 60 °C ± 2 °C.

5.5 Atomic absorption spectrometer, equipped with either "peak height" mode and printer, or "continuous" mode and pen recorder (full-scale response in 0,2 s), together with appropriate hollow cathode tube or electrodeless discharge lamp and deuterium background corrector.

NOTE 6 A Zeeman atomic absorption spectrometer is suitable.[1]

5.6 Graphite furnace atomizer, placed in the atomic absorption spectrometer (5.5), equipped with a control unit for temperature programming.

5.7 Graphite tube, uncoated.

NOTE 7 In combination with a pyrolytic platform, a pyrolytically coated graphite tube may be used.

5.8 Pyrolytic platform (optional).

6 Sampling

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

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

7 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

Do not filter the test sample.

8 Procedure

NOTE 8 If it is required to check whether the repeatability requirement is met, carry out two single determinations under repeatability conditions.

8.1 Preparation of apparatus

8.1.1 Switch on the atomic absorption spectrometer (5.5) and the deuterium background corrector.

8.1.2 In accordance with the manufacturer's instructions, adjust the lamp intensity, the slit, the wavelength and the amplification.

The required wavelength is 283,3 nm.

8.1.3 Optimize the position of the graphite furnace atomizer (5.6) in the atomic absorption spectrometer (5.5) and set the required programme on the control unit of the furnace, in accordance with table 1.

NOTE 9 If the apparatus available cannot be regulated in accordance with table 1, use a comparable programme suitable for the apparatus. If in this case a satisfactory background correction cannot be achieved, dilute the blank, the standard working solution and the test sample with an organic oil solvent (e.g. *n*-heptane) to a maximum of 1:2 (*m/m*) and carry out the spectrometric measurements at ambient temperature.

Place the platform (5.8), if used, in the graphite tube (5.7).

NOTE 10 Both atomization off the wall and atomization off the platform can be used. However, the accuracy and the sensitivity are twice as high with the latter type of atomization.

Table 1 — Programmes for the graphite furnace atomizer

Graphite tube	Step	Temperature °C	Ramp time s	Hold time s	Internal gas flow ml/min
Without platform	1	100	10	20	300
	2	650	60	40	300
	3	1 900	0	5	0
	4	2 700	1	3	50
With platform	1	200	10	20	300
	2	650	60	40	300
	3	1 700	0	5	0
	4	2 700	1	3	50

8.1.4 Before each injection of a sample, pretreat the pipette tip (5.3) by pipetting and then discarding 20 µl of cyclohexane.

NOTE 11 The film of cyclohexane remaining on the wall of the tip facilitates a reproducible transfer of the sample.

8.2 Pretreatment of test sample and prepared solutions

8.2.1 Place the test sample, the dilution oil (4.2) and the standard working solutions (4.4) in the oven (5.4), set at 60 °C, for at least 15 min before the determination.

8.2.2 Shake all test samples and solutions vigorously before analysis.

8.3 Determination

8.3.1 Graphite tube blank

Record the absorbance, if any, of the graphite tube and adjust the apparatus so that this reading corresponds to zero absorbance.

8.3.2 Dilution oil blank

Weigh 5,00 g of the pretreated dilution oil (8.2.1) in a bottle (5.1), add 5,00 g of the matrix modifier (4.6) and mix carefully.

Inject 20 µl of the mixture into the graphite furnace, initiate the programme and record the absorbance.

8.3.3 Standardization of apparatus

Weigh 5,00 g of each of the pretreated standard working solutions (8.2.1) in three bottles (5.1), add 5,00 g of the matrix modifier (4.6) to each and mix carefully.

Inject 20 µl of one of the three mixtures into the graphite furnace, initiate the programme and record the absorbance. Repeat this procedure for the other two mixtures.

8.3.4 Analysis of test sample

Weigh 5,00 g of the pretreated test sample (8.2.1) in a bottle (5.1), add 5,00 g of the matrix modifier (4.6) and mix carefully.

Inject 20 µl of this pretreated test sample into the graphite furnace, initiate the programme and record the absorbance.

8.3.5 Plotting the calibration graph

Draw a calibration curve by plotting the absorbance of each standard working solution (8.3.3), corrected for the blank (8.3.2), against their lead contents (in milligrams per kilogram).

NOTE 12 Sophisticated apparatus with auto-calibration may be used.

9 Expression of results

9.1 Measure the peak height on the recorder-chart or take readings on the display or printer.

9.2 Using the calibration graph, determine the lead content of the sample.

Express the result in milligrams per kilogram.

10 Precision

The precision of the method has been established by an international interlaboratory test organized by the International Union of Pure and Applied Chemistry (IUPAC) in 1988 and carried out in accordance with ISO 5725. In this test 20 laboratories participated and samples were investigated of soyabean oil and cocoa butter.

For the values obtained for the repeatability limit and the reproducibility limit, a probability level of 95 % holds.

10.1 Repeatability

The absolute difference between two 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, should not be greater than the value of r given in table 2.

Reject both results if the difference exceeds the value of r and carry out two new single determinations.

10.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the value of R given in table 2.

11 Test report

The test report shall specify

- the method in accordance with which sampling was carried out (if known),
- the method used,
- the test result(s) obtained, and
- if the repeatability has been checked, the final quoted result 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 2 — Repeatability limit r and reproducibility limit R

Values in milligrams per kilogram

Lead content	Sample	r	R
$w_{Pb} \leqslant 0,1$	Oil	$0,19\overline{w}_{Pb}$	$0,30\overline{w}_{Pb}$
	Fat	$0,15\overline{w}_{Pb}$	$0,68\overline{w}_{Pb}$

NOTE — $\overline{w}_{Pb}$ is the mean of the two results, in milligrams per kilogram.

Annex A
(informative)

Bibliography

[1] ISO 5555:1991, *Animal and vegetable fats and oils — Sampling.*

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