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Animal and vegetable fats and oils — Determination of copper, iron and nickel contents — Graphite furnace atomic absorption method

Corps gras d'origines animale et végétale — Détermination de la teneur en cuivre, fer et nickel — Méthode par spectrométrie d'absorption atomique avec four en graphite

Reference number
ISO 8294:1994(E)

Foreword

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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 8294 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.

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Animal and vegetable fats and oils — Determination of copper, iron and nickel contents — Graphite furnace atomic absorption method

1 Scope

This International Standard specifies a method for the determination of trace amounts of copper, iron and nickel 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 organo compounds of the metals under test. Calculation of the metal contents from the absorbances at selected wavelengths.

4 Reagents

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

4.1 Aluminium oxide, analytical grade.

NOTE 1 A suitable aluminium oxide (Quality No. 1077) is available from Merck, D-1600 Darmstadt, Germany.[1]

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

Store the oil in a metal-free polyethylene or polyethylene bottle (5.1). Metal contents of the oil shall be not greater than:

Cu, 3 µg/kg;

Fe, 5 µg/kg; and

Ni, 5 µg/kg.

NOTE 2 A sample of near-metal-free oil may be obtained by the following procedure.

Dissolve the oil in light petroleum (boiling range 40°C to 60 °C) in a ratio of 1 kg of oil to 3 litres of light petroleum. Prepare an aluminium oxide column (diameter/height ratio = 1/10) using twice the mass of aluminium oxide (activated by heating at 150 °C for 14 h) as the mass of the oil to be purified. Add the solution to the column and elute a volume of light petroleum 5/3 times as much as the volume the sample was dissolved in. Evaporate the light petroleum from the eluate on a heated water-bath using a gentle stream of nitrogen (2 l/min to 5 l/min). Remove the final traces of light petroleum under vacuum.

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[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.

4.3 Standard stock solutions

By appropriate dilution of organometallic standards with the dilution oil (4.2), prepare standard stock solutions containing respectively:

Cu, 2 mg/kg;

Fe, 10 mg/kg; and

Ni, 10 mg/kg.

NOTE 3 Suitable standards are 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 the following concentrations, by diluting the stock solutions (4.3) with the dilution oil (4.2):

Cu: 0,05 mg/kg; 0,1 mg/kg; 0,2 mg/kg

Fe: 0,25 mg/kg; 0,5 mg/kg; 1,0 mg/kg

Ni: 0,25 mg/kg; 0,5 mg/kg; 1,0 mg/kg

4.5 Niobium solution [$Nb(NO_3)_5$], containing 1 000 mg of niobium per litre; "AAS standard solution in aqueous matrix".

NOTE 4 A suitable solution is available from Alfa Division, 152 Andover Street, Danvers, MA 01923, USA (code 88083).[1)]

4.6 *n*-Heptane, analytical grade.

4.7 Argon, minimum purity 99,99 %.

NOTE 5 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 apparatus and, in particular, the following.

5.1 Bottles and **caps**, made of polyethylene or polypropylene, metal-free, of 20 ml and 50 ml capacity.

NOTE 6 If necessary, the bottles and caps may be rendered metal-free by cleaning thoroughly with warm nitric acid solution [$c(HNO_3) = 1$ mol/l], rinsing with water and then drying in an oven at about 80 °C.

5.2 Micropipettes, of 20 µl and 100 µl capacity.

5.3 Pipette tips

5.4 Electric oven, capable of being maintained at 150 °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.

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

5.7 Graphite tube, uncoated for atomization.

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

Determine each metal content one after the other.

NOTE 7 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 wavelengths appropriate for each metal are as follows:

— copper: 324,7 nm

— iron: 302,1 nm

— nickel: 232,0 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 8 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 solutions (4.4) and the test samples with an organic fat solvent (e.g. *n*-heptane) to a maximum of 1:2 (*m*/*m*) and carry out the spectrometric measurements at ambient temperature.

For the determination of copper and nickel content, use an uncoated graphite tube (5.7). For the determination of iron content, use a tube coated inside with niobium to ensure that the total amount of iron is determined.

NOTE 9 With an uncoated tube the results will vary according to the type of iron compound present in the fat. A suitable coating procedure is the following.

Inject 100 µl of niobium solution (4.5) into the furnace. Start the temperature programme to dry at 100 °C for 60 s and then atomize at 2 700 °C for 5 s. Repeat this procedure until 300 µl of niobium solution has been injected. Atomize at 2 700 °C until there is constant absorbance (to remove any iron contamination).

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

NOTE 10 The film of *n*-heptane remaining on the wall of the tip facilitates a reproducible transfer of the sample.

8.2 Pretreatment of test samples and prepared solutions

8.2.1 Place all test samples, 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 If the metal content of the crude fat is known to be outside the range given in 4.4, dilute it with the dilution oil (4.2).

8.2.3 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

Inject 20 µl of the dilution oil (4.2) into the graphite furnace, initiate the temperature programme and record the absorbance.

8.3.3 Standardization of apparatus

Inject 20 µl of one of the three standard working solutions of the metal under investigation (4.4) into the graphite furnace and record the absorbance. Repeat this procedure with the other two standard working solutions.

8.3.4 Analysis of test samples

8.3.4.1 Liquid samples at 40 °C

Inject 20 µl of the pretreated test sample (8.2) into the graphite furnace, initiate the temperature programme and record the absorbance.

Table 1 — Programmes for the graphite furnace atomizer

Determination	Step	Temperature °C	Ramp time s	Hold time s	Internal gas flow ml/min
For Cu	1 2	900 2 700	50 1	30 5	300 50
For Fe, Ni	1 2	1 200 2 700	50 1	30 5	300 50

8.3.4.2 Solid samples at 40 °C

Besides steps 1 and 2, introduce the following extra step in the programme on the control unit: hold time, 20 s; temperature, 60 °C; internal gas flow, 0 ml/min. Initiate the programme. During step 1 of the programme, inject 20 µl of the pretreated test sample (8.1) into the graphite furnace, allowing the tip to remain in the injection opening to liquify the fat and then injecting. Record the absorbance.

NOTE 11 The detection limit can be lowered either by expansion of the scale or by repeated injection of the sample at the end of step 1 and then allowing the programme to proceed to completion. If the metal content is too high (i.e. exceeds the range of the calibration curve) the test sample (8.2) should be diluted with the dilution oil (4.2) (see also 8.2.2).

8.3.5 Plotting the calibration graph

Draw a calibration curve for each of the metals by plotting the absorbance of each standard working solution (8.3.3), corrected for the blank (8.3.2) against the respective metal content (in milligrams per kilogram).

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

9 Expression of results

9.1 For each sample, measure the peak height on the recorder-chart or take readings on the display or printer.

9.2 Using the appropriate calibration graph, determine the metal contents of each sample, taking into account any dilution of the sample.

Express the results 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 1984 and carried out in accordance with ISO 5725.

In this test, 32 laboratories participated and samples of soyabean oil, groundnut oil and cocoa butter were investigated. Copper and iron were determined in both liquid and solid samples. Nickel was not determined in the cocoa butter since it is unlikely to occur in it.

For the values obtained for the repeatability and reproducibility limits, 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 calculated 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 3.

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 results 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 limits, r

Values in milligrams per kilogram

Element	Metal content, w	Sample	r
Cu	$w_{Cu} < 0,2$	Oil Fat	$0,010 + 0,14\overline{w}_{Cu}$ $0,008 + 0,11\overline{w}_{Cu}$
Fe	$w_{Fe} < 1,0$	Oil Fat	$0,077 + 0,08\overline{w}_{Fe}$ $0,026 + 0,20\overline{w}_{Fe}$
Ni	$w_{Ni} < 1,0$	Oil	$0,056 + 0,13\overline{w}_{Ni}$

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

Table 3 — Reproducibility limit, R

Values in milligrams per kilogram

Element	Metal content	Sample	R
Cu	$w_{Cu} < 0,2$	Oil Fat	$0,008 + 0,36\overline{w}_{Cu}$ $0,003 + 0,49\overline{w}_{Cu}$
Fe	$w_{Fe} < 1,0$	Oil Fat	$0,040 + 0,48\overline{w}_{Fe}$ $0,031 + 0,54\overline{w}_{Fe}$
Ni	$w_{Ni} < 1,0$	Oil	$0,027 + 0,44\overline{w}_{Ni}$

NOTE — $\overline{w}$ is the mean of both 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.*

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