INTERNATIONAL STANDARD

ISO 10540-2

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Animal and vegetable fats and oils — Determination of phosphorus content —

Part 2: Method using graphite furnace atomic absorption spectrometry

Corps gras d'origines animale et végétale — Détermination de la teneur en phosphore —

Partie 2: Méthode par spectrométrie d'absorption atomique avec four en graphite

Reference number
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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 10540-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

ISO 10540 consists of the following parts, under the general title *Animal and vegetable fats and oils — Determination of phosphorus content*:

— *Part 1: Colorimetric method*

— *Part 2: Method using graphite furnace atomic absorption spectrometry*

— *Part 3: Method using inductively coupled plasma (ICP) optical emission spectroscopy*

Animal and vegetable fats and oils — Determination of phosphorus content —

Part 2: Method using graphite furnace atomic absorption spectrometry

1 Scope

This International Standard specifies a rapid method for the determination of trace amounts of phosphorus ($\leqslant$ 40 mg/kg) in crude or refined animal and vegetable fats and oils (hereafter referred to as fats). The limit of determination of the method is 1,0 mg/kg.

2 Normative references

The following referenced documents are indispensable for the application of this document. 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 10540-1, *Animal and vegetable fats and oils — Determination of phosphorus content — Part 1: Colorimetric method*

3 Principle

A test portion is mixed with a matrix modifier then vaporized in a graphite furnace atomic absorption spectrometer, previously calibrated using standard dilutions of soya lecithin. The phosphorus content is calculated from the absorption at a wavelength of 213,5 nm.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

4.1 **Cyclohexane**.

4.2 **Matrix modifier**.

Dissolve 1 g of lanthanum organometallic standard with a lanthanum content of 5 000 mg/kg in 10 ml of cyclohexane (4.1).

It is essential that the lanthanum be added for the determination of the total phosphorus content. If it is omitted, the amount of phosphorus determined will vary, depending on the type of phosphatides present in the sample.

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

4.3 Blank oil: refined liquid edible oil with a phosphorus content below 1 mg/kg. The phosphorus content of the blank oil may be determined in accordance with ISO 10540-1.

4.4 Soya lecithin (commercial product)**,** well-defined, with a phosphorus content of about 2 % (by mass).

4.5 Standard stock solution, with a phosphorus content of about 400 mg/kg.

Dissolve 1 g of soya lecithin (4.4) in 4 g cyclohexane (4.1) and add 45 g of the blank oil (4.3). Determine the phosphorus content in accordance with ISO 10540-1.

4.6 Standard working solutions, with a phosphorus content of 10 mg/kg, 20 mg/kg and 40 mg/kg respectively. Dilute the stock solution (4.5) as appropriate with the blank oil (4.3).

NOTE A solution with a phosphorus content of 1 mg/kg may be useful when analysing palm oil or palm fractions.

4.7 Argon, of purity 99,98 %.

If argon is not available, nitrogen may be used as purge gas but it gives lower sensitivity.

WARNING — At temperatures above 2 300 °C nitrogen forms toxic hydrogen cyanide gas, therefore continuous ventilation shall be provided in the furnace area.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 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 electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector.

NOTE A Leeman atomic absorption spectrometer may be used.[1)]

5.2 Graphite furnace atomizer, placed in the atomic absorption spectrometer (5.1), equipped with a control unit for temperature programming.

5.3 Graphite tube, uncoated, with or without a pyrolytical platform.

In combination with a platform, a pyrolytically coated graphite tube may be used as well.

5.4 Micropipettor, of capacity 20 µl.

5.5 Pipettor tips.

5.6 Test tubes, of capacity 10 ml.

5.7 Oven, capable of being maintained at 60 °C ± 2 °C.

5.8 Autosampler for graphite furnace atomizer (optional), with polyethylene sample cups.

1) These are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 10540 and does not constitute an endorsement by ISO of these products.

6 Sampling

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

Sampling is not a part of the method specified in this part of ISO 10540. A recommended sampling method is given in ISO 5555.

Store samples in glass or poly(ethylene terephthalate) (PET) bottles.

7 Preparation of test sample

Prepare the test sample in accordance with ISO 661, ensuring that any sediment, which may be rich in phosphorus, is incorporated.

8 Procedure

8.1 Preparation of apparatus

8.1.1 Switch on the atomic absorption spectrometer (5.1) and the deuterium background corrector.

8.1.2 In accordance with the manufacturer's instructions, adjust the lamp current, the slit, the wavelength and the amplification. The required wavelength for phosphorus is 213,5 nm.

8.1.3 Optimize the position of the graphite furnace atomizer (5.2) in the atomic absorption spectrometer (5.1) and set the required programme on the control unit of the furnace. See Table 1.

Table 1 — Programme for the graphite furnace programmer

Step	Temperature °C	Ramp time s	Hold time s	Gas flow ml/min
1	600	40	20	300
2	1 600	50	40	300
3	2 800	0	5	0
4	2 800	1	3	50

If this programme cannot be realized, use a comparable programme suitable for the particular apparatus.

Place the platform, if used, in the graphite tube (5.3).

Both atomization off the wall and atomization off the platform may be used.

8.1.4 Before each injection of a sample solution, pretreat the pipettor tip (5.5) by pipetting and then discarding 20 µl of cyclohexane. The film of cyclohexane remaining on the wall of the tip facilitates a reproducible transfer of the sample solution.

Inject 20 µl of the standard working solution with phosphorus content of 40 mg/kg (4.6) into the graphite furnace using the micropipettor (5.4). Initiate the programme and record the absorption. Repeat these instructions until constant absorption has been reached.

Each time a graphite tube is replaced, carry out three determinations with the same standard working solution (4.6) to achieve equilibrium.

8.2 Preconditioning of test sample, blank oil and working solutions

At least 15 min before the determination, place the test sample (Clause 7), the blank oil (4.3) and the standard working solutions (4.6) in the oven (5.7). Shake each container vigorously immediately before further analysis.

If the phosphorus content is greater than 40 mg/kg, dilute the sample with blank oil (4.3). In that case, multiply the observed absorption with the dilution factor.

8.3 Determination

8.3.1 Graphite tube blank

Record the absorption, if any, of the graphite tube as such and auto-zero this absorption.

8.3.2 Blank oil

Weight, to the nearest 0,01 g, 1,00 g of the preconditioned blank oil (4.3) in a test tube (5.6). Add 1,00 g of matrix modifier (4.2) and mix thoroughly.

Inject 20 µl of this mixture into the graphite furnace using the micropipettor (5.4). Initiate the programme and record the absorption.

8.3.3 Calibration of the apparatus

Weigh, to the nearest 0,01 g 1,00 g of each of the three preconditioned standard working solutions (4.6) in three test tubes (5.6). To each test tube, add 1,00 g of matrix modifier (4.2) and mix thoroughly.

Inject 20 µl of the mixture into the graphite furnace using the micropipettor (5.4). Initiate the programme and record the absorption. Repeat until the absorption is constant.

8.3.4 Test sample

Weigh, to the nearest 0,01 g, 1,00 g of the preconditioned test sample in a test tube (5.6). Add 1,00 g of matrix modifier (4.2) and mix thoroughly.

Inject 20 µl of the mixture into the graphite furnace using the micropipettor (5.4), initiate the programme and record the absorption.

9 Expression of results

Measure the peak height on the recorder-chart or take the reading of the display or printer.

Draw a calibration curve by plotting the absorption of the three standard working solutions (8.3.3), corrected for the blank (8.3.2), against their phosphorus contents.

Read the phosphorus content of the sample from the calibration curve. Express the result in milligrams per kilogram.

NOTE With the use of sophisticated equipment, autocalibration can be applied.

10 Precision

10.1 Interlaboratory test

Details of 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 concentration ranges and matrices other than those given.

10.2 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 that 5 % of cases be greater than the repeatability limit (r), deduced by linear interpolation from Table 2.

10.3 Reproducibility

When the values of two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, lie within the range of the values in Table 1, the absolute difference between the two test results will in not more than 5 % of cases be greater than the reproducibility limit (R), deduced by linear interpolation from Table 2.

Table 2 — Repeatability and reproducibility limits at different phosphorus contents

Phosphorus content mg/kg	r mg/kg	R mg/kg
10	2,5	3,5
20	4,7	6,6
30	6,5	9,8

11 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 used, with reference to this part of ISO 10540;

— all operating details not specified in this part of ISO 10540, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result(s) obtained.

Annex A
(informative)

Results of interlaboratory tests

Two international tests were carried out on samples of sunflowerseed and soyabean oils using atomic absorption spectrometry.

The first international test was organized by the International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats and Derivatives in 1989. It was carried out in accordance with ISO 5725:1986. The final statistical analysis was carried out in accordance with ISO 5725-2:1994. In this test 21 laboratories participated, giving the precision data shown in Table A.1.

Table A.1 — Statistical results of the first interlaboratory test (1989)

Parameter	Sample [a]		
	A	B	C
Number of participating laboratories after eliminating outliers	17	17	17
Mean value, mg/kg	29,55	20,00	10,18
Repeatability standard deviation, s_r, mg/kg	2,30	1,69	0,91
Coefficient of variation of repeatability, %	7,8	8,4	9,0
Repeatability limit r ($r = 2{,}8\ s_r$), mg/kg	6,45	4,71	2,56
Reproducibility standard deviation, s_R, mg/kg	3,51	2,33	2,24
Coefficient of variation of reproducibility, %	11,9	11,7	12,2
Reproducibility limit R ($R = 2{,}8\ s_R$), mg/kg	9,82	6,63	3,48

[a] Sunflowerseed oils containing different levels of phosphorus:
A (high); B (medium); C (low).

The second interlaboratory test was organized by FOSFA International and the American Oil Chemists Society (AOCS), involving 13 laboratories. It was carried out in 1999. The results obtained are shown in Table A.2.

Table A.2 — Statistical results of the second interlaboratory test (1999)

Parameter	Sample [a]						
	A	B	C	D	E	F	G
Number of participating laboratories after eliminating outliers	5	5	5	5	5	5	5
Mean value, mg/kg	388,1	285,4	118,1	93,2	51,2	27,1	13,4
Repeatability standard deviation, s_r, mg/kg	3,3	15,6	1,9	12,0	1,1	1,0	0,3
Coefficient of variation of repeatability, %	1,4	5,5	1,6	12,8	2,1	3,5	2,2
Repeatability limit r ($r = 2{,}8\ s_r$), mg/kg	14,7	43,5	5,2	33,5	3,0	2,7	0,8
Reproducibility standard deviation, s_R, mg/kg	127,5	77,5	39,4	22,1	13,7	5,4	3,8
Coefficient of variation of reproducibility, %	32,9	27,2	33,4	23,7	26,7	19,4	28,6
Reproducibility limit R ($R = 2{,}8\ s_R$, mg/kg	356,9	216,9	110,4	61,8	38,3	15,0	10,8

[a] A to G: Samples of soyabean oil containing different levels of phosphorus.

The same samples were also tested using colorimetric method as specified in ISO 10540-1, and using inductively coupled plasma (ICP) optical emission spectrometry as specified in ISO 10540-3. The statistical results related to the colorimetric and ICP methods are presented in ISO 10540-1 and ISO 10540-3, respectively.