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

Part 1: Colorimetric method

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

Partie 1: Méthode colorimétrique

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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 10540-1 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 1: Colorimetric method

1 Scope

This part of ISO 10540 specifies a colorimetric method for the determination of the phosphorus content of animal and vegetable oils and fats.

This method is not suitable for determining the phosphorus content of commercial lecithin as this requires an ashing temperature of 800 °C.

2 Principle

The test portion is charred (carbonized) in the presence of magnesium hydroxycarbonate and then ashed. The ash is dissolved in dilute hydrochloric acid. The phosphorus content is then determined colorimetrically by the molybdenum blue method.

3 Reagents

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

3.1 Magnesium hydroxycarbonate, $[(MgCO_3)_n \cdot Mg(OH)_2] \cdot H_2O$, with a magnesium oxide content of between 40 % and 46 % (by mass).

Magnesium carbonate, hydrated, basic, $[(MgCO_3)_4 \cdot Mg(OH)_2] \cdot 5H_2O$, is suitable.

3.2 Hydrochloric acid, $c(HCl) = 2$ mol/l.

3.3 Sodium hydroxide solution, $c(NaOH) = 5$mol/l.

3.4 Reducing solution.

Weigh out 0,500 g of *p*-methylaminophenol sulfate $[(HOC_6H_4NHCH_3)_2 \cdot H_2SO_4]$, 2,5 g of sodium sulfite heptahydrate $(Na_2SO_3 \cdot 7H_2O)$ and 58,5g of sodium metabisulfite $(Na_2S_2O_5)$.

Transfer the weighed materials to a 1 litre volumetric flask. Dissolve in water, then dilute to the mark and mix. Keep the solution in a well-sealed brown bottle.

3.5 Sulfate/molybdate reagent.

Dissolve 25,0 g of ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 250 ml of 5 mol/l sulfuric acid [prepared by diluting 278 ml of concentrated (18 mol/l) sulfuric acid to 1 litre with water]. Transfer the solution to a 1 litre volumetric flask. Dilute to the mark with water, and mix. Store the solution in a brown bottle.

WARNING Care must be taken when diluting concentrated sulfuric acid.

3.6 Sodium acetate solution.

Dissolve 340 g of sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) in water. Transfer to a 1 litre volumetric flask. Dilute to the mark with water, and mix. Store the solution in a brown bottle.

3.7 Standard phosphate solution for calibration.

3.7.1 Stock solution (phosphorus content *ca*. 100 µg/ml).

Weigh, to the nearest 0,1 mg, about 440 mg of potassium dihydrogen phosphate (KH_2PO_4). Dissolve it in water and transfer quantitatively to a 1 litre volumetric flask. Dilute to the mark with water, and mix. Calculate the phosphorus content of the solution by the formula:

$$\rho = \frac{m_S \cdot M_P}{V \cdot M_S}$$

where

ρ is the phosphorus content of the stock solution, in micrograms per millilitre;

m_S is the mass of potassium dihydrogen phosphate, in milligrams;

M_P is the molar mass of phosphorus, in grams (M_P = 31,03 g);

V is the volume of stock solution in the flask, in litres (V = 1);

M_S is the molar mass of potassium dihydrogen phosphate, in grams (M_S = 136,09 g).

3.7.2 Standard phosphate solution 1 (phosphorus content *ca.* 10 µg/ml).

Pipette 25 ml of stock solution (3.7.1) into a 250 ml volumetric flask. Dilute to the mark with water, and mix. Calculate the phosphorus content of this solution by the formula:

$$\rho_{s1} = 0{,}1\ \rho$$

where

ρ_{s1} is the phosphorus content of the standard phosphate solution 1, in micrograms per millilitre;

ρ is the phosphorus content of the stock solution (3.7.1), in micrograms per millilitre.

3.7.3 Standard phosphate solution 2 (phosphorus content *ca.* 50 µg/ml).

Pipette 50 ml of stock solution (3.7.1) into a 100 ml volumetric flask. Dilute to the mark with water, and mix. Calculate the phosphorus content of this solution by the formula:

$$\rho_{s2} = 0{,}5\ \rho$$

where

ρ_{s2} is the phosphorus content of the standard phosphate solution 2, in micrograms per millilitre;

ρ is the phosphorus content of the stock solution (3.7.1), in micrograms per millilitre.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Test tubes, of 25 ml capacity, made of borosilicate glass, with stoppers and standard tapered necks, and graduated at 5 ml intervals or less. The reproducibility of the 15 ml graduation should be checked, as well as the resistance of the calibration marks to heating at 550 °C.

4.2 Block or **muffle furnace**, thermostatically controlled for temperatures up to 400 °C.

4.3 Ashing oven or **muffle furnace**, suitable for temperatures up to 700 °C.

4.4 Tube rack, resistant to high temperatures, preferably of corrosion-resistant steel, for use in the muffle furnace. The rack should hold the tubes at such an angle that the open ends are about 3 cm above the bottom of the tubes.

4.5 Spectrophotometer, suitable for measurements at 720 nm, using 1 cm and 4 cm cells.

4.6 Spectrophotometer cells, of path length 1 cm and 4 cm, and suitable for measurements at 720 nm.

5 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.

6 Preparation of test sample

If the sample is not completely liquid at room temperature, heat it to a maximum of 10 °C above the melting point. If the sample is not clear when liquid, homogenize it carefully immediately before weighing out the test portions. It is essential that any sediment, which may be rich in phosphorus, is incorporated homogeneously into the sample.

7 Procedure

7.1 Determination of the calibration factor

7.1.1 For phosphorus contents of 0 mg/kg to 125 mg/kg (in the oil)

Weigh 30 mg of magnesium hydroxycarbonate (3.1) into each of a series of seven test tubes (4.1). Using a microburette or pipette, add to the test tubes: 0 ml (blank); 0,25 ml; 0,5 ml; 1,0 ml; 1,5 ml; 2,0 ml; and 2,5 ml of standard phosphate solution 1 (3.7.2). The test tubes will contain quantities of phosphorus ranging from 0 µg to *ca.* 25 µg, equivalent to the amount of phosphorus in 0,2 g of an oil containing between 0 mg/kg to about 125 mg/kg of phosphorus.

NOTE The absorbance of the highest standard, containing 2,5 ml of standard phosphate solution 1 (under these conditions) should be approximately 0,8.

Add 2 ml of hydrochloric acid (3.2) to each test tube and wait until a clear solution is obtained. Then add 0,5 ml of sodium hydroxide solution (3.3) to each test tube and mix.

Using a pipette or burette, add 5 ml of reducing solution (3.4) to each test tube and mix.

In a similar manner, add 2,5 ml of sulfate/molybdate reagent (3.5) to each test tube, and mix. Stopper the tubes and let them stand for 20 min in a dark place.

Fill the test tubes to the 15 ml mark with sodium acetate solution (3.6), and mix.

Measure the absorbance of the solutions against the blank, in a 4 cm cell, at 720 nm.

Alternatively, measure the absorbance of all the solutions against water, as a check on the blank, and then, unless the equation of a regression line is to be computed, correct all the measurements for the blank value.

Calculate the calibration factor in accordance with 7.1.3. Alternatively, compute the equation of the regression line for the calibration (see 7.1.3.2).

7.1.2 For phosphorus contents of 125 mg/kg to 500 mg/kg (in the oil)

Weigh 30 mg of magnesium hydroxycarbonate (3.1) into each of a series of six test tubes. Using a microburette or pipette, add to the test tubes: 0 ml (blank); 0,5 ml; 0,8 ml; 1,2 ml; 1,6 ml; and 2,0 ml of standard phosphate solution 2 (3.7.3). The test tubes will contain quantities of phosphorus ranging from about 25 µg to 100 µg, equivalent to phosphorus contents of about 125 mg/kg to 500 mg/kg in the oil. The absorbance of the highest standard, in a 1 cm cell, should be approximately 0,8.

Add 2 ml of hydrochloric acid (3.2) to each test tube and wait until a clear solution is obtained. Then add 0,6 ml of sodium hydroxide solution (3.3) to each test tube, and mix.

Using a pipette or burette, add 5 ml of reducing solution (3.4) to each test tube, and mix.

In a similar manner, add 2,5 ml of sulfate/molybdate reagent (3.5) to each test tube, and mix. Stopper the test tubes and let them stand for 20 min in a dark place.

Fill the test tubes to the 15 ml mark with sodium acetate solution (3.6), and mix.

Measure the absorbance of the solutions against the blank, in a 1 cm cell, at 720 nm.

Alternatively, measure the absorbance of all the solutions against water, as a check on the blank, and then, unless the equation of a regression line is to be computed, correct all the measurements for the blank value.

Calculate the calibration factor in accordance with 7.1.3. Alternatively, compute the equation of the regression line for the calibration (see 7.1.3.2).

7.1.3 Calculation of the calibration factor

7.1.3.1 For each solution i of the series measured according to 7.1.1 and 7.1.2, calculate the calibration factor using the formula:

$$f_i = \frac{V_i \cdot \rho_S}{A_i}$$

where

f_i is the calibration factor for solution i of the series, in micrograms;

V_i is the volume of standard phosphate solution in solution i, in millilitres;

ρ_S is the phosphorus content of the phosphate solution used, in micrograms per millilitre;

A_i is the absorbance measured for solution i.

Use the average of the factors f_i as the calibration factor f for the calculation in Clause 8.

7.1.3.2 Alternatively, compute the equation of a regression line from all the optical density values measured against water, uncorrected for the blank value. The phosphorus content of the sample solution can then be calculated from this equation.

7.2 Ashing of oil sample

7.2.1 Weigh approximately 30 mg of magnesium hydroxycarbonate (3.1) into a test tube (4.1) and weigh the test tube, with the magnesium hydroxycarbonate, to the nearest 0,1 mg.

Using a Pasteur pipette, add approximately 0,2 g (10 to 15 drops) of the oil sample (Clause 6), taking care that all the sample material falls to the bottom of the tube and mixes with the magnesium hydroxycarbonate. No drops should be allowed to fall or splash onto the side walls of the test tube.

Reweigh the test tube to the nearest 0,1 mg.

Prepare a blank test tube containing magnesium hydroxycarbonate only.

7.2.2 Place the test tubes in the heating block or in the tube rack (4.4) in the cold muffle furnace (4.2). Heat the test tubes to 350 °C until the sample is carbonized to a dry black mass (1 h to 2 h).

After carbonization, increase the temperature to 550 °C and heat the sample at this temperature until the ash is completely white (about 2 h).

Remove the test tubes (and tube rack) and allow the test tubes to cool.

7.3 Colorimetric determination

Dissolve the residue from the ashing procedure in 2 ml of hydrochloric acid (3.2) by warming carefully until the liquid boils.

Allow the test tubes to cool and neutralize the contents by adding 0,6 ml of sodium hydroxide solution (3.3) to each. Then add, using a measuring pipette or burette, 5 ml of reducing solution (3.4) and mix.

In a similar manner, add 2,5 ml of sulfate/molybdate reagent (3.5), and mix.

Stopper the test tubes and allow them to stand in a dark place for 20 min.

Fill the test tubes to the 15 ml mark with sodium acetate solution (3.6), and mix.

Measure the absorbance of the solution against the blank in a 4 cm cell at 720 nm.

Alternatively, measure the absorbance of all the solutions against water, as a check on the blank, and then correct all the measurements for the blank value.

If the measured absorbance is higher than that of the highest standard (about 0,8), it lies outside the calibration range and the measurements shall be repeated, using a 1 cm cell.

8 Calculation

Calculate the phosphorus content using the formula:

$$w_P = \frac{f \cdot A}{m}$$

where

w_P is the phosphorus content of the test sample, in milligrams per kilogram;

f is the average calibration factor calculated as in 7.1.3, in micrograms;

A is the absorbance measured as in 7.3;

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

Alternatively, if the equation of a regression line is used for the calculation of the phosphorus content of the test portion then:

$$w_P = \frac{m_P}{m}$$

where

w_P is the phosphorus content of the oil, in milligrams per kilograms;

m_P is the phosphorus content of the test portion (7.2), in micrograms;

m is the mass of the test portion (7.2), in grams.

9 Precision

9.1 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.

9.2 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 not in more that 5 % of cases be greater than the repeatability limit (r), deduced by linear interpolation from Table 1.

9.3 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 1.

Table 1 — Repeatability and reproducibility limits at different phosphorus contents

Phosphorus content mg/kg	r mg/kg	R mg/kg
10	2	6
50	8	18
100	12	41
300	13	105
400	34	135

10 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 that 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 soyabean oil using the colorimetric method.

The first interlaboratory test was organized by the Federation of Oils, Seeds and Fats Associations (FOSFA) involving eight laboratories in four countries. It was carried out in July 1995. The results obtained were subjected to statistical analysis in accordance with ISO 5725:1986, giving the precision data shown in Table A.1.

Table A.1 — Statistical results of the first interlaboratory test (1995)

Parameter	Sample [a]	
	A	B
Number of participating laboratories after eliminating outliers	8	8
Mean value, mg/kg	10,51	318,31
Repeatability standard deviation, s_r, mg/kg	0,68	4,41
Coefficient of variation of repeatability, %	6,47	1,39
Repeatability limit r ($r = 2{,}8\ s_r$), mg/kg	1,92	12,34
Reproducibility standard deviation, s_R, mg/kg	2,12	17,93
Coefficient of variation of reproducibility, %	20,17	5,63
Reproducibility limit R ($R = 2{,}8\ s_R$), mg/kg	5,96	50,21

[a] Sample A: RBD soyabean oil.
Sample B: crude water-degummed soyabean oil.