International Standard 3946

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Starches and derived products — Determination of total phosphorus content — Spectrophotometric method

Amidons, fécules et produits dérivés — Détermination de la teneur en phosphore total — Méthode spectrophotométrique

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Foreword

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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.

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It has been approved by the member bodies of the following countries :

Austria	Netherlands	USA
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France	Portugal	
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Starches and derived products — Determination of total phosphorus content — Spectrophotometric method

1 Scope and field of application

This International Standard specifies a spectrophotometric method for the determination of the total phosphorus content of starch, including derivatives and by-products, in which the expected content, calculated as phosphorus (P), does not exceed 5 % (*m/m*).

2 Definition

total phosphorus content : The quantity of phosphorus determined in accordance with the conditions specified in this International Standard and expressed as phosphorus (P) as a percentage by mass of the product as received.

3 Principle

Destruction of the organic substances by digestion with a sulpho-nitric mixture and conversion of phosphates to orthophosphates.

Formation, by means of a reducing agent, of a phosphomolybdate know as molybdenum blue.

Spectrophotometric measurement of the intensity of the blue colour at a wavelength of 825 nm.

4 Reagents

During the analysis, use only reagents of recognized analytical grade, and only distilled water of at least equivalent purity.

4.1 Sulpho-nitric reagent.

Prepared by mixing 1 part by volume of sulphuric acid, ϱ_{20} 1,84 g/ml, 96 % (*m/m*) solution, and 1 part by volume of nitric acid, ϱ_{20} 1,38 g/ml, 65 % (*m/m*) solution.

4.2 Nitric acid, ϱ_{20} 1,38 g/ml, 65 % (*m/m*) solution.

4.3 Ascorbic acid, 50 g/l solution.

Keep this solution in a refrigerator for a maximum of 48 h.

4.4 Ammonium molybdate solution, prepared as follows.

In a 1 l flask, dissolve 10,6 g of ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 500 ml of water.

Add 500 ml of 10 mol/l sulphuric acid solution, mix and allow to cool to ambient temperature.

4.5 Sodium hydroxide, 10 mol/l solution.

4.6 Phosphorus, standard solutions.

4.6.1 Stock solution, corresponding to 100 mg of P per litre.

Weigh, to the nearest 0,5 mg, 0,439 3 g of anhydrous potassium dihydrogenorthophosphate and dissolve in water. Transfer quantitatively into a 1 000 ml one-mark volumetric flask.

Dilute to the mark with water and mix.

1 ml of this standard solution contains 100 µg of P.

NOTE — The potassium dihydrogenorthophosphate shall be dried before use for 1 h, in a drying oven controlled at 105 ± 2 °C, and then allowed to cool in a desiccator.

4.6.2 Standard solution, corresponding to 4 mg of P per litre.

Using a pipette, take 10 ml of the stock solution (4.6.1) and place it in a 250 ml one-mark volumetric flask.

Dilute to the mark with water and mix.

1 ml of this standard solution contains 4 µg of P.

5 Apparatus

NOTE — Ensure that the detergents used for cleaning glassware do not contain phosphorus.

Ordinary laboratory apparatus, and in particular

5.1 One-mark volumetric flasks, of capacities 50 - 100 - 200 - 250 and 500 ml, complying with the requirements of ISO 1042.

5.2 **Conical flasks**, of capacity 50 ml.

5.3 **Digestion flasks**, of capacity 100 ml.

5.4 **Pipettes**, of capacities 1 - 2 - 5 - 10 - 15 and 25 ml, complying with the requirements of ISO 648.

5.5 **Circulation-type cooling bath**, of temperature between 15 and 25 °C.

5.6 **Boiling water bath**.

5.7 **Hot-plate**.

5.8 **Desiccator**, containing an effective desiccant.

5.9 **Spectrophotometer**, fitted with cells of optical path length 1,0 cm, capable of measuring at a wavelength of 825 nm.

5.10 **Analytical balance**.

6 Procedure

Carry out the preparation of the calibration curve and the determination within 2 h.

6.1 Preparation of the calibration graph

Take a series of seven 50 ml conical flasks (5.2). Using a pipette (5.4), introduce into six of them 1,0 - 2,0 - 3,0 - 4,0 - 5,0 and 10,0 ml of the standard phosphorus solution (4.6.2), corresponding to 4 - 8 - 12 - 16 - 20 and 40 µg of P.

Add water to each of the seven flasks so that the total volume is approximately 30 ml. Mix.

Using a pipette, add to each of the flasks, in the following order, 4 ml of the ammonium molybdate solution (4.4) and 2 ml of the ascorbic acid solution (4.3). Mix after each addition.

Place the seven flasks in the boiling water bath (5.6) for 10 min.

Cool to ambient temperature by immersing the flasks in the cooling bath (5.5). Transfer the contents of the flasks quantitatively to the 50 ml one-mark volumetric flasks (5.1). Dilute to the marks with water and mix.

Using the spectrophotometer (5.9), determine the absorbance at 825 nm of each of the six solutions, using the solution from the flask without the standard solution as the reference. Plot the calibration curve giving the number of micrograms of phosphorus as a function of the absorbance.

6.2 Preparation of the test sample

Mix the sample thoroughly.

6.3 Test portion

Weigh, to the nearest 0,2 mg, 0,5 g of the test sample (6.2). This mass corresponds to an absorbance range between 0,1 and 0,7; if this is not the case, adjust the test portion accordingly (see the table).

6.4 Digestion

Transfer the test portion (6.3) into a digestion flask (5.3). Add 15 ml of the sulpho-nitric mixture (4.1) and mix well. Place the flask on the hot-plate (5.7). Heat gradually until the liquid is boiling gently in the flask. Continue boiling until the brown vapour is replaced by white vapour and the liquid has become clear.

A persistent dark colour can be eliminated by adding the nitric acid solution (4.2), drop by drop, whilst continuing the digestion.

Allow to cool, then add 10 ml of water and eliminate the excess nitric acid solution by heating until the flask is again filled with white vapour.

6.5 Preparation of the test solution

Cool the mixture (6.4) again and add 45 ml of water. Raise the pH to 7 with the sodium hydroxide solution (4.5). Transfer the contents of the digestion flask to a one-mark volumetric flask of suitable volume (5.1). Dilute to the mark with water. Mix thoroughly.

6.6 Determination

Take an aliquot portion (see the table) and introduce it into a 50 ml conical flask.

Using a pipette, add, in the following order, 4 ml of the ammonium molybdate solution (4.4) and 2 ml of the ascorbic acid solution (4.3). Mix after each addition.

Place the flask in the boiling water bath (5.6) for 10 min.

Cool to ambient temperature by immersing the flask in the cooling bath (5.5). Transfer quantitatively to a 50 ml one-mark volumetric flask (5.1). Dilute to the mark with water and mix.

Using the spectrophotometer, determine the absorbance at 825 nm of this solution.

Read from the calibration curve (see 6.1) the corresponding number of micrograms of phosphorus.

6.7 Blank test

Carry out a blank test in parallel with the determination, replacing the test portion by water.

6.8 Number of determinations

Carry out two determinations on the same test sample (6.2).

7 Expression of results

7.1 Method of calculation and formula

The total phosphorus (P) content, expressed as a percentage by mass, is given by the formula

$$\frac{m_1 \times V_0 \times 100}{m_0 \times V_1 \times 10^6}$$

where

m_0 is the mass, in grams, of the test portion (6.3);

m_1 is the phosphorus content, in micrograms, of the test solution read from the calibration curve (6.1);

V_0 is the dilution volume, in millilitres, of the test solution (6.5);

V_1 is the volume, in millilitres, of the aliquot portion used for the determination (6.6).

Take as the result the arithmetic mean of the values obtained in the two determinations provided that the conditions of repeatability (7.2) are satisfied. If not, repeat the test.

7.2 Repeatability

The difference between the values obtained in two determinations carried out simultaneously by the same analyst on the same test sample (6.2), shall not exceed

— 2 % of their arithmetic mean in the case of phosphorus contents greater than 0,2 % (m/m);

— 0,004 g of phosphorus per 100 g of product in cases of phosphorus contents less than 0,2 % (m/m).

8 Test report

The test report shall indicate the method used and the results obtained. In addition, it shall mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have affected the results.

The test report shall include all details required for the complete identification of the sample.

Table

Expected phosphorus content % (m/m)	**Mass of test portion** (see 6.3) g	**Dilution volume** (see 6.5) ml	**Volume of the aliquot portion** (see 6.6) ml
< 0,05	0,500	100	25
0,05 to 0,10	0,500	100	10
0,10 to 0,25	0,500	100	2
0,25 to 0,50	0,500	200	2
0,50 to 1,00	0,250	250	2
1,00 to 2,00	0,125	250	2
2,00 to 5,00	0,125	500	2

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