International Standard 6491

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Animal feeding stuffs — Determination of total phosphorus content — Spectrophotometric method

Aliments des animaux — Détermination de la teneur en phosphore total — Méthode spectrophotométrique

First edition — 1980-08-15

UDC 636.085/.087 : 543.42 : 546.18 **Ref. No. ISO 6491-1980 (E)**

Descriptors : animal feeding, animal nutrition, chemical analysis, determination of content, phosphorus, spectrophotometric analysis.

Price based on 3 pages

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

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.

International Standard ISO 6491 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in February 1979.

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

Australia	Hungary	Poland
Austria	India	Portugal
Brazil	Israel	Romania
Canada	Kenya	South Africa, Rep. of
Chile	Korea, Rep. of	Spain
Cyprus	Libyan Arab Jamahiriya	Thailand
Czechoslovakia	Malaysia	Turkey
Egypt, Arab Rep. of	Netherlands	United Kingdom
Ethiopia	New Zealand	Yugoslavia
France	Philippines	

No member body expressed disapproval of the document.

Animal feeding stuffs — Determination of total phosphorus content — Spectrophotometric method

1 Scope

This International Standard specifies a spectrophotometric method for the determination of the total phosphorus content of animal feeding stuffs.

2 Field of application

The method is applicable to all animal feeding stuffs.

It is particularly appropriate for the analysis of products low in phosphorus. In certain cases (products rich in phosphorus) a gravimetric method may be used.

3 Principle

Ashing of a test portion, either by dry combustion and dissolution in acid (in the case of organic feeding stuffs), or by acid digestion (in the case of mineral compounds and liquid feeding stuffs).

Treatment of this solution with molybdovanadate reagent and measurement of the absorbance of the yellow solution thus obtained, in a spectrophotometer, at 430 nm.

4 Reagents

All reagents shall be of recognized analytical quality. Distilled water or water of at least equivalent purity shall be used.

4.1 Calcium carbonate.

4.2 Hydrochloric acid, $c(HCl) \approx 6$ mol/l.

4.3 Nitric acid, $c(HNO_3) \approx 1$ mol/l.

4.4 Nitric acid, ϱ_{20} 1,38 g/ml.

4.5 Sulphuric acid, ϱ_{20} 1,84 g/ml.

4.6 Molybdovanadate reagent.

In a 1 000 ml volumetric flask, mix 200 ml of the ammonium heptamolybdate solution (4.6.1), 200 ml of the ammonium monovanadate solution (4.6.2) and 135 ml of the nitric acid (4.4). Make up to the mark with water.

4.6.1 Ammonium heptamolybdate solution.

Dissolve in hot water 100 g of ammonium heptamolybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}.4H_2O]$. Add 10 ml of ammonia ($\varrho_{20}$ 0,91 g/ml) and make up to 1 litre with water.

4.6.2 Ammonium monovanadate solution.

Dissolve 2,35 g of ammonium monovanadate (NH_4VO_3) in 400 ml of hot water.

Stirring constantly, slowly add 20 ml of diluted nitric acid [7 ml of the nitric acid (4.4) + 13 ml of water] and make up to 1 litre with water.

4.7 Phosphorus, standard solution containing 1 mg of phosphorus per millilitre.

In a 1 000 ml volumetric flask, dissolve 4,394 g of potassium dihydrogen phosphate (KH_2PO_4) previously dried at 103 °C, in water. Make up to the mark with water.

5 Apparatus

Usual laboratory apparatus, and in particular:

5.1 Ashing crucibles, of silica or porcelain.

5.2 Electric muffle-furnace, capable of being controlled at 550 ± 20 °C.

5.3 Kjeldahl flask, of capacity 250 ml.

5.4 One-mark volumetric flasks, of capacities 500 and 1 000 ml.

5.5 Spectrophotometer, fitted with 10 mm cells, suitable for measurements at 430 nm.

5.6 Glass test tubes, of capacity 25 to 30 ml, fitted with ground glass stoppers.

5.7 Sand bath.

5.8 Beaker, of capacity 250 ml.

5.9 Graduated pipettes.

5.10 Analytical balance.

6 Procedure

6.1 Test portion and preparation of the test solution

According to the nature of the sample, take a test portion and prepare the test solution as specified in 6.1.1 or 6.1.2.

6.1.1 Dry ashing (for samples containing organic substances and free from phosphates which give insoluble products on ashing)

Weigh about 2,5 g of the sample[1] to the nearest 1 mg in an ashing crucible (5.1).

Mix the test portion thoroughly with 1 g of the calcium carbonate (4.1). Ash in the furnace (5.2) at 550 ± 20 °C until white or grey ash is obtained (a small quantity of carbon does not interfere).

Transfer the ash to the 250 ml beaker (5.8).

Add 20 ml of water and then hydrochloric acid (4.2) until effervescence ceases.

Add a further 10 ml of the hydrochloric acid (4.2).

Place the beaker on the sand bath (5.7) and evaporate to dryness to render the silica insoluble.

Allow to cool.

Add 10 ml of the nitric acid (4.3) to the residue and boil on the sand bath for 5 min, without evaporating to dryness.

Decant the liquid into a 500 ml volumetric flask (5.4), rinsing the beaker several times with hot water.

Leave to cool, make up to the mark with water, mix and filter.

6.1.2 Wet ashing (for mineral compounds and liquid feeding stuffs)

Weigh 1 g or more of the sample[1] to the nearest 1 mg.

Place the test portion in the Kjeldahl flask (5.3), add 20 ml of the sulphuric acid (4.5), shake to impregnate the substance completely with acid and to prevent it from sticking to the wall of the flask, heat and keep at boiling point for 10 min.

Leave to cool slightly, add 2 ml of the nitric acid (4.4), heat gently, leave to cool slightly, add a little more nitric acid (4.4) and bring back to the boiling point.

Repeat this procedure until a colourless solution is obtained.

Cool, add a little water, and decant the liquid into a 500 ml volumetric flask (5.4), rinsing the Kjeldahl flask with hot water.

Leave to cool, make up to the mark with water, mix and filter.

6.2 Development of coloration and measurement of absorbance

Dilute an aliquot portion of the filtrate obtained (6.1.1 or 6.1.2) with water, to obtain a phosphorus concentration of not more than 40 µg/ml.

Transfer, by means of a pipette (5.9), 10 ml of this solution to a test tube (5.6) and add, by means of another pipette, 10 ml of the molybdovanadate reagent (4.6).

Mix and leave to stand for at least 10 min at 20 °C.

Transfer a portion of the solution obtained to a measuring cell and measure the absorbance in the spectrophotometer (5.5) at 430 nm, using as the reference liquid a solution obtained by adding 10 ml of molybdovanadate reagent (4.6) to 10 ml of water.

6.3 Number of determinations

Carry out two determinations on test portions taken from the same test sample.

6.4 Preparation of the calibration curve

6.4.1 Using the standard phosphorus solution (4.7), and by means of the graduated pipettes (5.9), prepare solutions containing respectively 5, 10, 20, 30 and 40 µg of phosphorus per millilitre.

6.4.2 Transfer, by means of a pipette (5.9), 10 ml of each of these solutions to a series of five test tubes (5.6) and add, to each by means of another pipette, 10 ml of the molybdovanadate reagent (4.6).

Mix and leave to stand for at least 10 min at 20 °C.

Measure the absorbance of each solution as specified in 6.2.

6.4.3 Draw the calibration curve by plotting the absorbances against the corresponding concentrations of phosphorus, in micrograms per millilitre, of the standard phosphorus solutions (6.4.1).

For concentrations between 0 and 40 µg/ml, the curve shall be linear.

1) The sampling and sample preparation of animal feeding stuffs will form the subject of future International Standards.

6.5 Blank test

Carry out a blank test in parallel with the determination, using the same procedure and the same quantities of all the reagents, but omitting the test portion.

7 Expression of results

7.1 Method of calculation and formula

The phosphorus content, expressed as a percentage by mass of the product as received, is equal to

$$\frac{X \times 500 \times F \times 100}{m \times 10^6} = \frac{X \times F}{20 \times m}$$

where

X is the phosphorus content, in micrograms per millilitre of the diluted aliquot portion of the test solution, read from the calibration curve (6.4.3);

m is the mass, in grams, of the test portion (6.1.1 or 6.1.2);

F is the reciprocal dilution factor for the aliquot portion (see 6.2).

Take as the result the arithmetic mean of the two determinations (see 6.3), provided that the conditions of repeatability (see 7.2) are satisfied.

Report the result to the nearest :

0,01 % (m/m) phosphorus for phosphorus contents less than 3 % (m/m);

0,1 % (m/m) phosphorus for phosphorus contents greater than or equal to 3 % (m/m).

7.2 Repeatability

The difference between the results of two determinations carried out in rapid succession by the same analyst shall not exceed :

3 % (relative value) of their mean for phosphorus contents less than 5 % (m/m);

0,15 (absolute value) for phosphorus contents greater than or equal to 5 % (m/m).

8 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

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