DRAFT INTERNATIONAL STANDARD
ISO/DIS 23776

ISO/TC **34**/SC **6** Secretariat: **SAC**

Voting begins on: **2020-12-02** Voting terminates on: **2021-02-24**

Meat and meat products — Determination of total phosphorous content

Viandes et produits à base de viande — Détermination de la teneur en phosphore

ICS: 67.120.10

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Reference number
ISO/DIS 23776:2020(E)

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Published in Switzerland

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

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This secondedition cancels and replaces the firstedition (ISO 13730:1996), which has been technically revised.

The main changes compared to the previous edition are as follows:

— A new test method - inductively coupled plasma optical emission spectrometry (ICP-OES) method is added.

— The clauses order of the document has been rearranged.

— The determination scope of the document has been modified.

— The title of the document has been modified.

— The introductory texts for “Foreword”, “Normative references” and “Terms and definitions” have been modified in accordance with the requirements of ISO/IEC Directives, Part 2.

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Meat and meat products — Determination of total phosphorous content

1 Scope

This International Standard specifies three methods for the determination of total phosphorous content - Inductively coupled plasma optical emission spectrometry (ICP-OES) method, Spectrometric method and Gravimetric method of all kinds of meat and meat products, including poultry and livestock.

For the inductively coupled plasma optical emission spectrometry (ICP-OES) method, the limit of detection (LOD) is 1.0mg/kg and limit of quantify (LOQ) is 3.0 mg/kg if the mass of test portion is 0.5g, constant volume is 50ml.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 936:1978, *Meat and meat products—Determination of ash*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

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3 Terms and definitions

3.1
total phosphorous content of meat and meat products
mass of phosphorous pentoxide determined by the procedure specified in this International Standard, expressed as a percentage of the mass of the test portion

4 Principle

4.1 Inductively coupled plasma optical emission spectrometry (ICP-OES) method

After digestion, the sample is determined by inductively coupled plasma emission spectrometer with the characteristic spectral line wavelength of phosphorous. In a certain concentration range, the spectral line signal intensity of phosphorous is proportional to its concentration, and is quantified by the standard curve method.

4.2 Spectrometric method

Drying of the test portion and incineration of the residue. After cooling, hydrolysis of the ash with nitric acid. Filtration and dilution followed by the formation of a yellow compound with a mixture of ammonium monovanadate and ammonium heptamolybdate. Photometric measurement at a wavelength of 430 nm.

4.3 Gravimetric method

Mineralization of a test portion with sulphuric and nitric acids. Precipitation of the phosphorous as quinoline phosphomolybdate. Drying and weighing of the precipitate. An alternative method of mineralization is described in clause 10.

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5 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 17604[1].

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

Start from a representative sample of at least 200 g. Store the sample in such a way that deterioration and change in composition are prevented.

6 Inductively coupled plasma optical emission spectrometry (ICP-OES) method

6.1 Reagents

Use only reagents of recognized analytical grade: G.R. Guarantee reagent.

Water: complying with at least grade 1 in accordance with ISO 3696.

6.1.1 Nitric acid (HNO3), GR or higher purity,ρ_{20}=1,40g/ml.

6.1.2 Argon (Ar): argon (> 99.995%) or liquid argon

6.1.3 Nitric acid (5 + 95): take 50mL nitric acid(6.1.1), slowly add 950mL water, mix.

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6.1.4 phosphorous standard stock solution (1000mg/L) is certified by the state and is awarded the standard solution of certified reference material. c (P) =1000 mg/l; c (P_2O_5) =2294 mg/l.

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This stock solution is stable for 1 month when stored in the dark.

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6.1.5 Standard series solution of phosphorous: accurate extract standard reserve liquid, dilute standard series solution with nitric acid solution (5+95). The mass concentration is 0mg/L, 20.0mg/L, 40.0mg/L, 60.0mg/L, 80.0mg/L, and 100.0mg/L respectively.

NOTE According to the sensitivity of the instrument and the actual content of phosphorous in the sample, the concentration range of the standard solution should be adjusted appropriately.

6.2 Apparatus

The usual laboratory equipment and, in particular, the following.

6.2.1 Inductively coupled plasma-optical emission spectrometer

6.2.2 Analytical balance, capable of weighing to the nearest 0.0001g.

6.2.3 Microwave digestion instrument: with polytetrafluoroethylene digestion internal tank

6.2.4 Electric hot plate with adjustable temperature control, or graphite digestion unit.

6.2.5 The ultrasonic water bath

6.2.6 Homogenizer, high speed pulverizer: capable of samole pulverizing and homogenizing.

6.2.7 One-mark volumetric flasks, of capacities 25±0.04 ml and 50±0.06ml

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6.2.8 One-mark pipettes, of capacities 2±0.02 mL, 5 ±0.05mL, 10 ±0.10mL .

6.2.9 Graduated (automatic) pipettes, of capacities 2 mL, 5 mL, 10 mL.

6.2.10 Polytetrafluoroethylene digestion tube

6.3 Procedure

6.3.1 Sample pre-treatment: Samples with low water content are mixed together after removing debris. The samples with high water content are homogenized.

6.3.2 Sample digestion

Microwave digestion: weigh, 0.2g~0.5g of the test portion (accurate to 0.001g, the sample with more moisture content can be appropriately increased to 1g~2g) in the microwave digestion internal tank (6.2.10) with Polytetrafluoroethylene digestion tube (6.2.10), add 5mL~10mL nitric acid(6.1.1), stand for 1h or overnight, screw the tank cap, follow the standard operation steps of the microwave digestion instrument to digest. Take out after cooling, slowly open the tank cap and vent, flush the inner cap with a little water, put the digestion tank on electric hot plate (with adjustable temperature control)(6.2.4) or in the ultrasonic water bath(6.2.5), heat 30min at 100°C or ultrasonic degassing for 2min~5min, dilute with water to 25mL or 50mL and mix, do blank test at the same time.

6.4 Determination

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6.4.1 Instrument reference conditions: optimize the operating conditions of the instrument; make the instrument sensitivity and other indicators to meet the requirements of the analysis. The reference conditions for the instrument operation are observation mode: horizontal observation; power: 1150W; plasma gas flow: 15L /min; auxiliary gas flow: 0.5L /min; atomized gas flow: 0.65L /min, measured line: 213.6nm, 214.9 nm, 178.3 nm, or177.4 nm (select one of them).

6.4.2 Standard curve drawing: the standard series working solution is injected into the inductively coupled plasma emission spectrometer, and the intensity signal response of the analytical spectral line is determined. When the element concentration is abscissa, the spectral line intensity response value is Y-axis, and the standard curve is drawn.

6.4.3 Test portion

Sample determination: the blank solution and sample solution are injected into the inductively coupled plasma emission spectrometer respectively. The signal response values of the spectral line strength are measured, and the concentration of phosphorous in the solution is obtained according to the standard curve.

6.5 Calculation and expression of results

Calculate the phosphorous.

Content using the following equation:

$$X = \frac{(\rho - \rho_0) \times V \times f}{m}$$

where

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X is the phosphorous content, mg/kg;

ρ is the phosphorous mass concentration of test portion, mg/L;

ρ_0 is the phosphorous mass concentration of blank test, mg/L;

V is the constant volume of sample digestion solution, ml;

m is the numerical value of the mass, in grams of test portion;

f is dilution factor;

Three bit valid numbers are reserved for the results of the calculation.

The results of the interlaboratory comparison in the test method will be attached soon

6.6 Limit of detection

The limit of detection (LOD) is 1.0mg/kg and limit of quantify (LOQ) is 3.0 mg/kg if the mass of test portion is 0.5g, constant volume is 50ml.

6.7 Precision

The precision of the method has been established by an interlaboratory test, carried out in accordance with ISO 5725 (ref. [2]).

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6.8 Repeatability

The absolute difference between two independent single test results, obtained using the same method on test material in the same laboratory by the same operator using the same equipment within a short interval of time (result see Annex A).

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6.9 Reproducibility

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, (result see Annex A).

7 Spectrometric method

7.1 Reagents

Use only reagents of recognized analytical grade and distilled or demineralised water or water of at least equivalent purity.

7.1.1 Nitric acid (HNO3), ρ_{20}=1,40g/ml. GR or higher purity

7.1.2 Nitric acid, 1+2 (v/v) dilutions.

Mix 1 volume of nitric acid [65 %(m/m); ρ_{20}=1, 40g/ml (7.1.1) with two volumes of water.

7.1.3 Ammonium meta-vanadate(Ammonium mono-vanadate) solution (NH_4VO_3), 2.5g/l.

Dissolve 2.5g of ammonium metavanadate in 500 ml of boiling water. Cool and add 20 ml of the nitric acid (7.1.2), dilute to the mark 1L with water and mix.

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7.1.4 Ammonium heptamolybdate solution, $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$, 50g/l (CAS 12027-67-7)

Dissolve 50g of ammonium heptamolybdate tetrahydrate in about 800 ml of warm water (at approx. 50°C). Cool and transfer quantitatively to a 1000ml volumetric flask. Dilute to the mark with water and mix.

7.1.5 Colour reagent

Mix one volume of the nitric acid (7.1.2) with one volume of the ammonium metavanadate solution (7.1.3). Subsequently add one volume of the ammonium heptamolybdate solution (7.1.4) and mix. Please make sure the order of addition.

7.1.6 Potassium dihydrogen phosphate (KH_2PO_4), (CAS: 7778-77-0, >99.99%) is certified by the state and is awarded the standard solution of certified reference material.

Phosphate stock solution, c (P) =109 mg/l; c (P_2O_5) =250 mg/l.

Dissolve in water 479.4 mg of potassium dihydrogen phosphate (KH_2PO_4), previously dried for 3 h at 103°C±2°C and allowed to cool in desiccators.

Transfer quantitatively to a 1000 ml volumetric flask. Dilute to the mark with water and mix.

7.1.7 Phosphate standard solutions, containing between 0.05 mg and 0.30 mg of P_2O_5 per millilitre.

Transfer by pipette or burette to 100 ml volumetric flasks 10 ml, 20 ml, 30 ml, 40 ml, 50 ml and 60 ml of the phosphate stock solution (7.1.6). Add 10 ml of the nitric acid (7.1.2). Dilute to the mark with water and mix.

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Note the range of standard curve can be adjusted according to the use of different concentrations of standard substance, use water to dilute.

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7.1.8 Blank solution.

Pipette 2 ml of the nitric acid (7.1.2) and 30 ml of the colour reagent (7.1.5) into a 100 ml volumetric flask. Dilute to the mark with water and mix.

7.2 Apparatus

Important: all glassware shall be thoroughly cleaned using a phosphate-free detergent and then rinsed with water.

Usual laboratory equipment and, in particular, the following.

7.2.1 Mechanical or electrical equipment capable of homogenizing the laboratory sample. This includes a high speed rotational cutter, or a mincer fitted with a plate with holes not exceeding 4.5 mm in diameter(see also clause).

7.2.2 Water bath, capable of being maintained at 100 °C.

7.2.3 Fluted filter paper, of diameter 15 cm, phosphate-free.

7.2.4 Spectrometer, capable of being used at a wavelength of 430 nm±2 nm, or a photo-electric colorimeter with an interference filter with absorption maximum at 430 nm±2 nm.

7.2.5 Glass cells, of 10 mm optical path length.

7.2.6 Analytical balance, capable of weighing to an accuracy of ±0.001 g.

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7.2.7 One-mark volumetric flasks, of capacities 100±0.10 ml and 1000±0.40 ml.

7.2.8 Muffle furnace, with adjustable temperature control, temperature (550±25) °C

7.2.9 Electric hot plate with adjustable temperature control

7.2.10 Porcelain crucible with 60mm diameter, 25mm high inclined wall

7.2.11 Desiccator, provided with and effective desiccant.

For details of this and other apparatus needed for the incineration procedure, see ISO 936.

7.2.12 Mechanical meat mincer, laboratory size, fitted with a plate with holes of diameter not exceeding 4mm.

7.3 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (7.2.1). Take care that the temperature of the sample material does not rise above 25°C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared test sample, close the container and store in such a way that deterioration and change in composition are prevented. Analyse the test sample as soon as practicable, but always within 24 h after homogenization.

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7.4 Procedure

NOTE If it is required to check whether the repeatability requirement (7.8) is met, carry out two single determinations in accordance with 7.4.1 to 7.5.

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7.4.1 Test portion

7.4.1.1 Weigh, to the nearest 0.001 g, about 5 g of the prepared test sample. Carry out the mineralization of the test portion (7.4.1) by using an incinerator (7.2.8) and the method described in ISO 936. Take up the resulting ash in 10 ml of the nitric acid (7.1.2) using a stirring rod to aid dissolution. Cover the dish with a watch glass and heat for 30 min on boiling water bath (7.2.2). Allow to cool and transfer the liquid quantitatively to a 100 ml volumetric flask (7.2.7). Dilute to the mark with water, mix and filter through the filter paper (7.2.3), rejecting the first 5 ml to 10 ml of filtrate.

7.4.2 Determination

Pipette 20 ml of the clear and colourless filtrate (7.4.1.1) into a 100ml volumetric flask (7.2.7) and add 30 ml of the colour reagent (7.1.5) by pipette. Dilute to the mark with water and mix. Allow to stand for at least 15 min.

7.4.2.1 Measure the absorbance at a wavelength of 430 nm ±2 nm in a glass cell (7.2.5) against the blank solution (7.1.8), using the spectrometer or the photo-electric colorimeter equipped with an interference filter (7.2.4).

7.4.2.2 Read the phosphorous concentration of the sample solution from the calibration graph obtained as described in 7.5.

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7.5 Calibration graph

7.5.1 Pipette 20 ml of each phosphate standard solution (7.1.7) into 100 ml volumetric flasks.

Add to these solutions 30 ml of the colour reagent. Dilute to the mark with water to obtain concentrations of 10µg, 20µg, 30µg, 40µg, 50µg and 60µg of P_2O_5 per millilitre, respectively. Mix and allow standing for at least 15 min.

7.5.2 Carry out the procedure described in 7.4.2.1

7.5.3 Plot the measured absorbance values, corrected for the blank value, against the concentrations of the diluted phosphate standard solutions (7.5.1). Construct the best-fitting straight line through the plotted points and the origin. The specified minimum correlation coefficient R^2 ≥0.95.

It is necessary to prepare a new calibration graph for each series of analyses.

NOTE If the color development of the sample exceeds the highest point of the standard curve, dilute the sample reasonably , and conduct the test in 7.4.2.

7.6 Calculation

Calculate the total phosphorous content, expressed as phosphorous pentoxide as percentage by mass of the test portion, by the formula:

$$x = \frac{c}{20m}$$

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where

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c is the phosphorous pentoxide concentration, in micrograms per millilitre, of the sample solution (7.4.2.4) as read from the calibration graph;

m is the mass, in grams, of the test portion (7.4.1).

Report the result rounded to two decimal places.

7.7 Precision

The precision of the method has been established by an interlaboratory test (see refs. [3.4]), only with processed sausages, carried out in accordance with ISO 5725 (ref. [2]).

7.8 Repeatability

The absolute difference between two independent single test results, obtained using the same method on 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 0.0070% *(m/m)*.

7.9 Reproducibility

The absolute difference between two independent 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 0.0117% *(m/m)*.

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