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**Meat and meat products —  
Determination of total phosphorous  
content**

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

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# Contents

	Page
Foreword.....	v
<b>1 Scope.....</b>	<b>1</b>
<b>2 Normative references.....</b>	<b>1</b>
<b>3 Terms and definitions.....</b>	<b>1</b>
<b>4 Principle.....</b>	<b>1</b>
4.1 Inductively coupled plasma optical emission spectrometry (ICP-OES) method.....	1
4.2 Spectrometric method.....	2
4.3 Gravimetric method.....	2
<b>5 Sampling.....</b>	<b>2</b>
<b>6 Inductively coupled plasma optical emission spectrometry (ICP-OES) method.....</b>	<b>2</b>
6.1 Reagents.....	2
6.2 Apparatus.....	2
6.3 Procedure.....	3
6.3.1 Sample pre-treatment.....	3
6.3.2 Sample digestion.....	3
6.4 Determination.....	4
6.4.1 Instrument reference conditions.....	4
6.4.2 Standard curve drawing.....	4
6.4.3 Test portion.....	4
6.5 Calculation and expression of results.....	4
6.6 Limit of detection.....	5
6.7 Precision.....	5
6.8 Repeatability.....	5
6.9 Reproducibility.....	5
<b>7 Spectrometric method.....</b>	<b>5</b>
7.1 Reagents.....	5
7.2 Apparatus.....	6
7.3 Preparation of test sample.....	7
7.4 Procedure.....	7
7.4.1 Test portion.....	7
7.4.2 Determination.....	7
7.5 Calibration graph photo-electric.....	7
7.6 Calculation.....	8
7.7 Precision.....	8
7.8 Repeatability.....	8
7.9 Reproducibility.....	8
<b>8 Gravimetric method.....</b>	<b>8</b>
8.1 Reagents.....	8
8.2 Apparatus.....	9
8.3 Procedures.....	9
8.3.1 Preparation of test sample.....	9
8.3.2 Test portion.....	9
8.3.3 Mineralization.....	10
8.3.4 Determination.....	10
8.3.5 Blank test.....	10
8.3.6 Expression of results.....	10
8.3.7 Repeatability.....	11
8.4 Notes on procedure.....	11
<b>9 Test report.....</b>	<b>11</b>
<b>Annex A (informative) International laboratory ring test (ICP-OES method).....</b>	<b>12</b>

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established 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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 (see [www.iso.org/directives](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

This first edition cancels and replaces ISO 2294:1974 and ISO 13730:1996, which have been technically revised. The main changes compared with ISO 2294:1974 and ISO 13730:1996 are as follows:

- a new test method, the inductively coupled plasma optical emission spectrometry (ICP-OES) method, has been added;
- the structure of the document has been revised;
- the title of the document has been modified;
- the Scope has been modified.

This corrected version of ISO 23776:2021 incorporates the following corrections:

- the concentration of phosphate stock solution (7.1.6) has been increased from  $c(\text{P}) = 109 \text{ mg/l}$ ;  $c(\text{P}_2\text{O}_5) = 250 \text{ mg/l}$  to  $c(\text{P}) = 218 \text{ mg/l}$ ;  $c(\text{P}_2\text{O}_5) = 500 \text{ mg/l}$ ;
- the amount of phosphate stock solution (7.1.6) dissolved in water has been increased from 479,4 mg to 958,8 mg.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).



# Meat and meat products — Determination of total phosphorous content

## 1 Scope

This document specifies three methods for the determination of the total phosphorous content of all kinds of meat and meat products, including poultry and livestock:

- the inductively coupled plasma optical emission spectrometry (ICP-OES) method;
- the spectrometric method;
- the gravimetric method.

For the ICP-OES method, the limit of detection (LOD) is 1,0 mg/kg and the limit of quantification (LOQ) is 3,0 mg/kg if the mass of the test portion is 0,5 g and the constant volume is 50 ml.

## 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, *Meat and meat products — Determination of total ash*

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

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## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

### 3.1

#### **total phosphorous content of meat and meat products**

mass of phosphorous pentoxide determined by the procedure specified in this document

Note 1 to entry: It is expressed as a percentage of the mass of the test portion.

## 4 Principle

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

The test portion of the sample is microwave digested with nitric acid. The concentration of phosphorous is determined by ICP-OES using external calibration. 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 sulfuric and nitric acids. Precipitation of the phosphorous as quinoline phosphomolybdate. Drying and weighing of the precipitate. An alternative method of mineralization is described in [8.4](#).

## 5 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 17604.

It is important that the laboratory receives 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. [ISO 23776:2021](#)

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**6.1.1** Water, conforming to at least grade 1 in accordance with ISO 3696.

**6.1.2 Nitric acid (HNO<sub>3</sub>)**, concentrated, not less than 65 % (mass fraction) or higher purity,  $\rho_{20} = 1,42$  g/ml.

**6.1.3 Argon (Ar)**: argon (> 99,995 %, mass fraction) or liquid argon.

**6.1.4 Nitric acid (5 + 95)**: take 50 ml nitric acid ([6.1.2](#)), slowly add 950 ml water and mix.

**6.1.5 Phosphorous standard stock solution (1 000 mg/l)**,  $c(\text{P}) = 1\ 000$  mg/l;  $c(\text{P}_2\text{O}_5) = 2\ 294$  mg/l.

This stock solution is stable for one month when stored in the dark at room temperature.

**6.1.6 Standard series solution of phosphorous**: accurate extract standard reserve liquid, dilute standard series solution with nitric acid solution (5 + 95). The mass concentration is 0 mg/l, 20,0 mg/l, 40,0 mg/l, 60,0 mg/l, 80,0 mg/l and 100,0 mg/l.

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

**IMPORTANT** — All glassware shall be thoroughly cleaned using a phosphate-free detergent and then rinsed with water.

The usual laboratory apparatus and, in particular, the following shall be used.



**6.2.1 Inductively coupled plasma optical emission spectrometer.**

**6.2.2 Analytical balance**, capable of weighing to the nearest 0,000 1 g.

**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 Ultrasonic water bath.**

**6.2.6 Homogenizer**, high-speed pulverizer, capable of sample pulverizing and homogenizing.

**6.2.7 One-mark volumetric flasks**, of capacities 25 ml and 50 ml.

**6.2.8 One-mark pipettes**, of capacities 2 ml, 5 ml and 10 ml.

**6.2.9 Graduated (automatic) pipettes**, of capacities 2 ml, 5 ml and 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 0,2 g to 0,5 g of the test portion (accurate to 0,001 g, the sample with more moisture content can be appropriately increased to 1 g to 2 g) in the microwave digestion internal tank (6.2.3) with the polytetrafluoroethylene digestion tube (6.2.10);
- add 5 ml to 10 ml of nitric acid (6.1.2);
- stand for 1 h or overnight;
- screw the tank cap;
- follow the standard operation steps of the microwave digestion instrument (6.2.3) 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 (6.2.3) on the electric hot plate (with adjustable temperature control) (6.2.4) or in the ultrasonic water bath (6.2.5);
- heat for 30 min at 100 °C or ultrasonic degassing (approximately 40 kHz) for 2 min to 5 min;
- dilute with water to 25 ml or 50 ml and mix;
- do a blank test at the same time.

## 6.4 Determination

### 6.4.1 Instrument reference conditions

The process is as follows:

- optimize the operating conditions of the instrument;
- ensure the instrument sensitivity and other indicators meet the requirements of the analysis;
- the reference conditions for the instrument operation are observation mode:
  - horizontal observation;
  - power: 1 150 W;
  - plasma gas flow: 15 l/min;
  - auxiliary gas flow: 0,5 l/min;
  - atomized gas flow: 0,65 l/min;
  - measured line (select one): 213,6 nm, 214,9 nm, 178,3 nm, 177 nm or 177,4 nm.

### 6.4.2 Standard curve drawing

The standard series working solution is injected into the inductively coupled plasma optical emission spectrometer (6.2.1) 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

For sample determination, the blank solution and sample solution are injected into the inductively coupled plasma optical emission spectrometer (6.2.1). 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,  $X$ , in milligrams per kilogram, using [Formula \(1\)](#):

$$X = \frac{(\rho - \rho_0) \times V \times f}{m} \quad (1)$$

where

$\rho$  is the phosphorous mass concentration of the test portion, in milligrams per litre;

$\rho_0$  is the phosphorous mass concentration of the blank test, in milligrams per litre;

$V$  is the constant volume of the sample digestion solution, in millilitre;

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

$f$  is the dilution factor.

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

## 6.6 Limit of detection

The LOD is 1,0 mg/kg and the LOQ is 3,0 mg/kg if the mass of the test portion is 0,5 g and the constant volume is 50 ml.

## 6.7 Precision

The precision of the method was established by an international laboratory ring test, carried out in accordance with ISO 5725-2.

## 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 (see [Annex A](#)).

## 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 (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 (HNO<sub>3</sub>),**  $\rho_{20} = 1,42$  g/ml, guarantee reagent or higher purity.

**7.1.2 Nitric acid:** water 1:2 (volume/volume).

Mix one volume of nitric acid [mass fraction of 65 %;  $\rho_{20} = 1,42$  g/ml ([7.1.1](#))] with two volumes of water.

**7.1.3 Ammonium metavanadate (ammonium monovanadate) solution (NH<sub>4</sub>VO<sub>3</sub>),** 2,5 g/l.

Dissolve 2,5 g 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 1 l with water and mix.

**7.1.4 Ammonium heptamolybdate solution, [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O],** 50 g/l (CAS 12027-67-7).

Dissolve 50 g of ammonium heptamolybdate tetrahydrate in about 800 ml of warm water (at approximately 50 °C). Cool and transfer quantitatively to a 1 000 ml 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. Make sure of the order of addition. It can be kept stable in the dark for one month.

**7.1.6 Phosphate stock solution,** c(P) = 218 mg/l; c(P<sub>2</sub>O<sub>5</sub>) = 500 mg/l.

Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), (CAS: 7778-77-0, > 99,99 %).