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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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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 (see www.iso.org/directives).

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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.

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.

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.

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*

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.

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

6.1.2 Nitric acid (HNO_3), concentrated, not less than 65 % (mass fraction) or higher purity, ρ_{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(P) = 1 000 mg/l; $c(P_2O_5)$ = 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):

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

where

ρ is the phosphorous mass concentration of the test portion, in milligrams per litre;

ρ_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_3), ρ_{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 %; ρ_{20} = 1, 42 g/ml (7.1.1)] with two volumes of water.

7.1.3 Ammonium metavanadate (ammonium monovanadate) solution (NH_4VO_3), 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, [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$], 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, $c(P)$ = 109 mg/l; $c(P_2O_5)$ = 250 mg/l.

Potassium dihydrogen phosphate (KH_2PO_4), (CAS: 7778-77-0, > 99,99 %).

Dissolve in water 479,4 mg of potassium dihydrogen phosphate (KH_2PO_4), previously dried for 3 h at (103 ± 2) °C and allowed to cool in desiccators.

Transfer quantitatively to a 1 000 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.

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

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.

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

7.2.1 Mechanical or electrical equipment capable of homogenizing the laboratory sample, including a high speed rotational cutter or a mincer fitted with a plate with holes not exceeding 4,5 mm in diameter.

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 ± 2) nm, or a **photo-electric colorimeter** with an interference filter with absorption maximum of (430 ± 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.

7.2.7 One-mark volumetric flasks, of capacities (100 ± 0,10) ml and (1 000 ± 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 60 mm diameter, 25 mm high inclined wall.

7.2.11 Desiccator, provided with an 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 4 mm.

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. The storage time should be not more than 24 h.

7.4 Procedure

7.4.1 Test portion

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

7.4.1.2 Weigh, to the nearest 0,001 g, about 5 g of the prepared test sample. Carry out the mineralization of the test portion 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 a 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

7.4.2.1 Pipette 20 ml of the clear and colourless filtrate (7.4.1.1) into a 100 ml 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.

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

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

7.5 Calibration graph photo-electric

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 10 µg, 20 µg, 30 µg, 40 µg, 50 µg and 60 µg of P_2O_5 per millilitre. Mix and allow to stand 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 determination coefficient $R^2 \geq 0{,}95$.

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

If the colour 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, *x*, expressed as phosphorous pentoxide as percentage by mass of the test portion, using Formula (2):

$$x = \frac{c}{20m} \tag{2}$$

where

c is the phosphorous pentoxide concentration, in micrograms per millilitre, of the sample solution 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 was established by an interlaboratory test (see References [4] and [5]) only with processed sausages, carried out in accordance with ISO 5725-2.

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 a mass fraction of 0,007 0 %.

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 a mass fraction of 0,011 7 %.

8 Gravimetric method

8.1 Reagents

All reagents shall be of recognized analytical reagent quality. Distilled water or water of equivalent purity shall be used in the test.

8.1.1 Sulfuric acid, ρ_{20} = 1,84 g/ml.

8.1.2 Nitric acid, ρ_{20} = 1,40 g/ml.

8.1.3 Precipitating reagent.

8.1.3.1 Dissolve 70 g of sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$) in 150ml of water.

8.1.3.2 Dissolve 60 g of citric acid monohydrate [CH_2 (CO_2H) COH (CO_2H) CH_2 (CO_2H)·H_2O] in 150 ml of water and add 85 ml of nitric acid (8.1.2).

8.1.3.3 Gradually add solution 8.1.3.1 to solution 8.1.3.2 while stirring.

8.1.3.4 To 100 ml of water add successively 35 ml of nitric acid (8.1.2) and 5 ml of distilled quinoline.

Gradually add this solution to the mixture 8.1.3.3 while stirring. Leave for 24 h at room temperature.

Filter (using quantitative filter paper, fast), add 280 ml of acetone and dilute to 1 000 ml with water.

Store the reagent in a well-stoppered plastic bottle in the dark.

8.2 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.

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

8.2.2 Analytical balance with an accuracy of ±0,001 g.

8.2.3 Kjeldahl flask, 250 ml capacity, or a **long-necked round-bottomed flask**.

8.2.4 Heating device, on which the flask (8.2.3) can be heated in an inclined position in such a way that the source of heat only touches the part of the wall of the flask which is below the level of the liquid.

8.2.5 Suction device, to remove the acid fumes evolved during the digestion.

8.2.6 Fritted glass filter, pore diameter 5 µm to 15 µm.

8.2.7 Electrically heated drying oven, with temperature control, which can be adjusted to (260 ± 20) °C.

8.2.8 Conical suction flask.

8.2.9 Desiccator, provided with an effective desiccant.

8.2.10 Pasteur pipette.

8.3 Procedures

8.3.1 Preparation of test sample

Make the sample homogeneous by passing it at least twice through the meat mincer (8.2.1) and mixing. Keep the homogeneous sample in a completely filled, air-tight, closed container and store it in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as possible, but in any case within 24 h, according to the method given in 8.3.2 to 8.3.4, or 8.4.

If the sample is not immediately analysed after passing through the mincer, liquid separation can occur on standing. Therefore, homogenize the test sample thoroughly with a fork immediately before taking the test portion.

8.3.2 Test portion

Weigh, to the nearest 0,001g, about 3 g of the test sample into the flask (8.2.3). See also the note to 8.4.

8.3.3 Mineralization

Add 20 ml of nitric acid (8.1.2) and some glass beads or boiling chips.

Place the flask in an inclined position (at an angle of about 40 ° from the vertical) on the heating device (8.2.4). Heat for 5 min, cool and then add 5 ml of sulfuric acid (8.1.1).

Heat the flask gently until the foaming has ceased. Then heat somewhat more strongly. As soon as the mixture starts to carbonize, add more nitric acid by means of a Pasteur pipette (8.2.10) and continue until the brown fumes have ceased.

Finally, when the liquid has become colourless, heat until white fumes appear.

Cool, add 15 ml of water and boil gently for 10 min, minimizing the evaporation of water (e.g. by means of a pear-shaped glass bulb inserted in the neck of the flask).

Transfer the liquid quantitatively into a 250 ml beaker or conical flask, rinsing the flask (8.2.3) with water. Add 10 ml of nitric acid. The total liquid volume should then be about 50 ml.

8.3.4 Determination

Add 50 ml of precipitating reagent (8.1.3) to the liquid in the beaker or conical flask.

Cover with a watch glass and boil for 1 min on a hotplate placed in the suction device (8.2.5).

Allow to cool to room temperature. During cooling, swirl the contents three or four times.

Filter under suction through the fritted glass filter (8.2.6), which has been previously heated for 30 min at a temperature of (260 ± 20) °C, cooled in the desiccators (8.2.9) and weighed to the nearest 1 mg.

Wash the precipitate on the filter five times with 25 ml portions of water, using this water, at the same time, to wash any remaining precipitate from the conical flask onto the filter.

Dry in the oven (8.2.7) at a temperature of (260 ± 20) °C for 1 h.

Cool in the desiccators (8.2.9) and weigh to the nearest 1 mg.

Carry out two determinations on the same test sample.

If the mass of the dried precipitate is more than 750 mg, repeat the analysis with a smaller test portion.

8.3.5 Blank test

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

8.3.6 Expression of results

Calculate the phosphorous content, expressed as percentage phosphorous pentoxide by mass, using Formula (3):

$$0{,}032\,07 \times m_1 \times \frac{100}{m_0} = 3{,}207 \times m_1 / m_0 \tag{3}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in grams, of the quinoline phosphomolybdate precipitate.

Take as the result the arithmetic mean of the two determinations, provided that the conditions of repeatability are satisfied (see 8.3.7).

Report the result to the nearest 0,01 g of phosphorous pentoxide per 100 g of sample.

8.3.7 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not be greater than 0,02 g of phosphorous pentoxide per 100 g of the sample.

8.4 Notes on procedure

If desired, the mineralization can be carried out by incineration using the method described in ISO 936. Then proceed as follows:

— take up the ash in 15 ml of concentrated nitric acid (8.1.2), using a stirring rod to aid dissolution;

— transfer the liquid to a 250 ml conical flask;

— wash the ashing dish and the stirring rod several times with water and add the washings to the contents of the conical flask;

— dilute to about 75 ml;

— heat for 30 min in a boiling water bath;

— allow to cool, and proceed according to 8.3.4.

9 Test report

The test report shall specify:

— the sample;

— the International Standard used (including its year of publication);

— the method used (if the standard includes several);

— the result, including a reference to the clause which explains how the results were calculated;

— any deviations from the procedure;

— any unusual features observed;

— the date of the test.

Annex A
(informative)

International laboratory ring test (ICP-OES method)

A.1 Overview

The international laboratory ring test of this document was conducted from March 2020 to June 2020. Fourteen laboratories participated in two parallel tests of five samples each.

The test was conducted by the Shanghai Institute of Quality Inspection and Technical Research, China, which also prepared the statistical analysis and final report.

The ICP-OES test method described in this document is adopted here for the determination of phosphorous content in meat product samples.

Five different kinds of meat samples (A: beef; B: chicken; C: duck; D: mutton; and E: pork) were used during the test, each with several mean levels.

The precision of the test results was evaluated based on ISO 5725-2.

A.2 Statistical analyses of the test results of phosphorous content

A.2.1 Original test results

Fourteen laboratories participated in the determination of phosphorous content in meat samples. The results are shown in Table A.1.

Table A.1 — Original test results of the determination of phosphorous content

Laboratory *i*	Level *j* mg/kg									
	A		B		C		D		E	
1	2,24E+03	2,22E+03	4,09E+03	4,08E+03	2,22E+03	2,21E+03	4,02E+03	4,00E+03	3,64E+03	3,66E+03
2	2,28E+03	2,32E+03	4,29E+03	4,24E+03	2,23E+03	2,21E+03	4,21E+03	4,16E+03	3,73E+03	3,74E+03
3	2,32E+03	2,30E+03	4,20E+03	4,22E+03	2,24E+03	2,28E+03	4,08E+03	4,02E+03	3,78E+03	3,70E+03
4	2,36E+03	2,52E+03	4,35E+03	4,44E+03	2,36E+03	2,38E+03	4,37E+03	4,25E+03	3,95E+03	3,99E+03
5	2,28E+03	2,29E+03	4,16E+03	4,22E+03	2,21E+03	2,21E+03	3,82E+03	4,09E+03	3,65E+03	3,65E+03
6	2,11E+03	2,11E+03	3,95E+03	3,92E+03	1,80E+03	1,82E+03	3,52E+03	3,51E+03	3,48E+03	3,48E+03
7	2,17E+03	2,15E+03	4,13E+03	4,12E+03	2,13E+03	2,20E+03	3,94E+03	3,87E+03	3,61E+03	3,63E+03
8	2,18E+03	2,17E+03	3,96E+03	4,01E+03	2,10E+03	2,09E+03	3,81E+03	3,81E+03	3,42E+03	3,45E+03
9	2,28E+03	2,35E+03	4,22E+03	4,22E+03	2,26E+03	2,26E+03	4,07E+03	4,08E+03	3,74E+03	3,76E+03
10	2,08E+03	2,10E+03	3,70E+03	3,81E+03	1,95E+03	1,98E+03	3,58E+03	3,73E+03	3,30E+03	3,25E+03
11	2,13E+03	2,07E+03	4,11E+03	4,03E+03	2,07E+03	2,06E+03	3,83E+03	3,79E+03	3,51E+03	3,55E+03
12	2,03E+03	2,01E+03	3,79E+03	3,76E+03	1,88E+03	1,93E+03	3,65E+03	3,75E+03	3,32E+03	3,41E+03
13	2,09E+03	2,09E+03	3,91E+03	3,83E+03	2,00E+03	2,01E+03	3,66E+03	3,69E+03	3,44E+03	3,37E+03
14	2,45E+03	2,39E+03	4,22E+03	4,28E+03	2,24E+03	2,19E+03	4,13E+03	4,15E+03	3,72E+03	3,76E+03

A.2.2 Cell means

The cell means of the determination of phosphorous content are shown in Table A.2.

Table A.2 — Cell means of the determination of phosphorous content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	2,23E+03	4,09E+03	2,22E+03	4,01E+03	3,65E+03
2	2,30E+03	4,27E+03	2,22E+03	4,19E+03	3,74E+03
3	2,31E+03	4,21E+03	2,26E+03	4,05E+03	3,74E+03
4	2,44E+03	4,40E+03	2,37E+03	4,31E+03	3,97E+03
5	2,29E+03	4,19E+03	2,21E+03	3,96E+03	3,65E+03
6	2,11E+03	3,94E+03	1,81E+03	3,52E+03	3,48E+03
7	2,16E+03	4,13E+03	2,17E+03	3,91E+03	3,62E+03
8	2,18E+03	3,99E+03	2,10E+03	3,81E+03	3,44E+03
9	2,32E+03	4,22E+03	2,26E+03	4,08E+03	3,75E+03
10	2,09E+03	3,76E+03	1,97E+03	3,66E+03	3,28E+03
11	2,10E+03	4,07E+03	2,07E+03	3,81E+03	3,53E+03
12	2,02E+03	3,78E+03	1,91E+03	3,70E+03	3,37E+03
13	2,09E+03	3,87E+03	2,01E+03	3,68E+03	3,41E+03
14	2,42E+03	4,25E+03	2,22E+03	4,14E+03	3,74E+03

A.2.3 Cell absolute differences

The cell absolute differences of the determination of phosphorous content are shown in Table A.3.

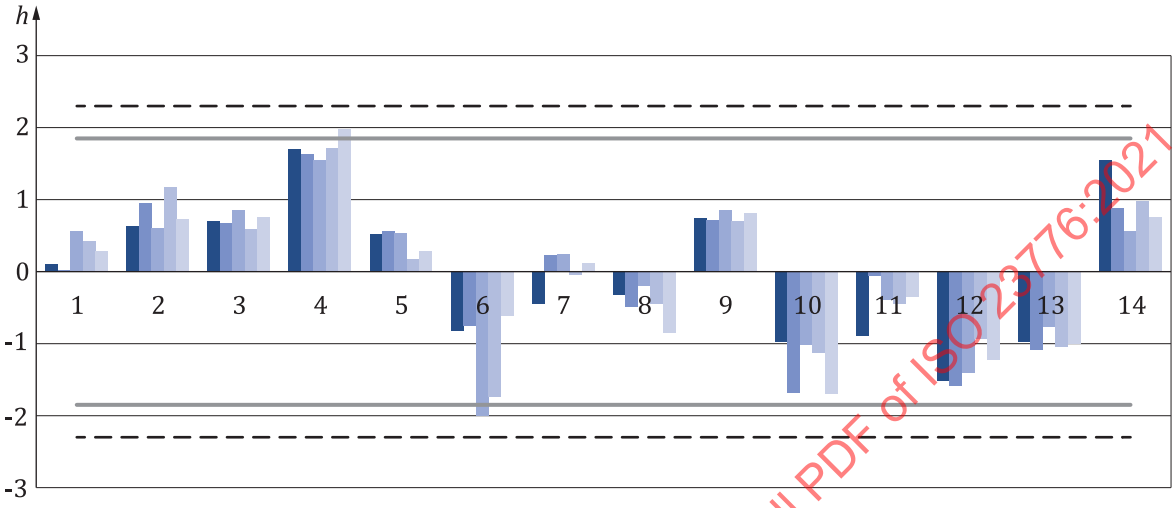
Table A.3 — Cell absolute differences of the determination of phosphorous content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	20,0	10,0	10,0	20,0	20,0
2	40,0	50,0	20,0	50,0	10,0
3	20,0	20,0	40,0	60,0	80,0
4	160	90,0	20,0	120	40,0
5	10,0	60,0	0	270	0
6	0,00	30,0	20,0	10,0	0
7	20,0	10,0	70,0	70,0	20,0
8	10,0	50,0	10,0	0	30,0
9	70,0	0	0	10,0	20,0
10	20,0	110	30,0	150	50,0
11	60,0	80,0	10,0	40,0	40,0
12	20,0	30,0	50,0	100	90,0
13	0	80,0	10,0	30,0	70,0
14	60,0	60,0	50,0	20,0	40,0

A.2.4 Scrutiny of results for consistency and outliers

A.2.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

Calculate the interlaboratory consistency statistic, *h*, as well as the intralaboratory consistency statistic, *k*, for each level of each laboratory. Plot the *h* and *k* values for each cell in order of laboratory to get Mandel's *h* and *k* graphs, see Figures A.1 and A.2.

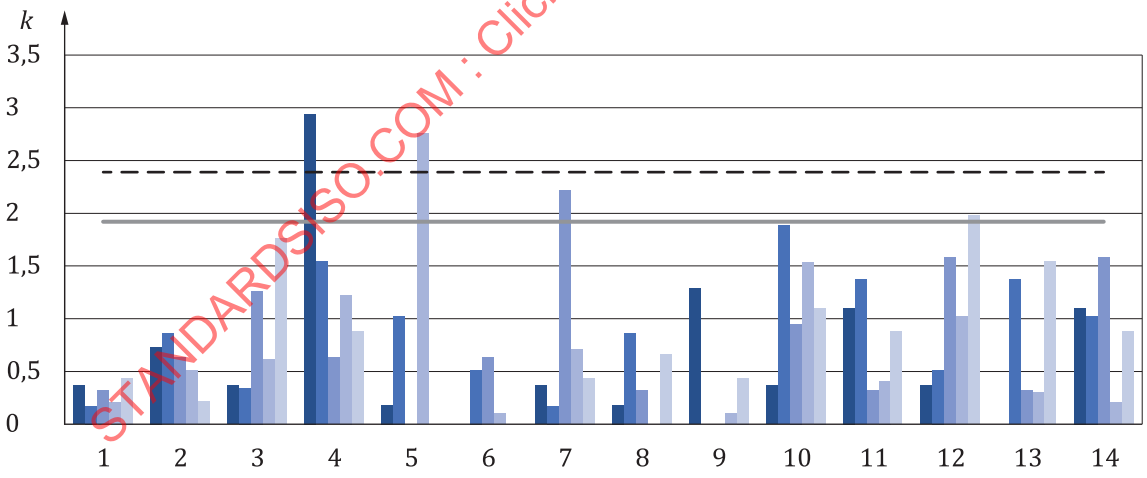


Key

1 % critical values

5 % critical values

Figure A.1 — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure A.2 — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 6 had a straggler on level C, as did laboratory 4 on level E, while no outlier has been found herein.