International Standard

ISO 5553

Second edition
2024-02

Meat and meat products — Detection of condensed phosphates

Viandes et produits à base de viande — Recherche des phosphates condensés

Reference number
ISO 5553:2024(en)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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.

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

This second edition cancels and replaces the first edition (ISO 5553:1980), which has been technically revised.

The main changes are as follows:

— term “polyphosphates” has been revised to “condensed phosphates”;

— clauses have been reordered;

— Clause 5 “Sampling” has been updated;

— Clause 7 “Reagents and Apparatus” has been updated;

— the amount of extracting solution has been revised and the temperature conditions of the extracting solution has been deleted (8.2.1);

— “Limit of detection” has been added (8.5);

— “Repeatability and reproducibility” has been added (8.6);

— Clause 9 “Interpretation” has been modified and the R_F values of the phosphates in the reference mixture have been revised;

— “Annex A” and “Annex B” have been added;

— “Bibliography” has been added.

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 — Detection of condensed phosphates

1 Scope

This document specifies a method for the detection of linear condensed phosphates in meat and meat products by thin layer chromatographic separation.

This document only applies to the detection of added condensed phosphates that are still present in the sample at the time of investigation, because condensed phosphates are gradually hydrolyzed by enzymes present in meat or meat products and during heat treatment of meat or meat products.

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

3 Terms and definitions

No terms and definitions are listed in this document.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

Extraction of the meat or meat products with trichloroacetic acid. Clearing of the serum obtained with ethanol/diethyl ether mixture. Separation of the phosphates by thin layer chromatography and detection of condensed phosphates by spraying with reagents for colour development.

5 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in CAC/GL 50-2004.

It is important that the laboratory-received sample 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 Preparation of the test sample

Homogenize the sample by using the appropriate equipment (7.2.5). If using a mechanical meat grinder with plate, run the sample through the machine at least twice. Keep it in a completely filled, air-tight, closed

container and store it, if necessary, in a refrigerator. Analyse the sample as soon as possible, but in any case, within 5 h. If the serum cannot be prepared immediately, store the sample at -20 °C for 2 months.

7 Reagents and apparatus

7.1 Reagents

All reagents shall be of recognized analytical quality. Water of at least grade 3 in accordance with ISO 3696 shall be used.

Warning — All appropriate safety precautions shall be observed when carrying out the procedures specified in this document.

7.1.1 Isopropyl alcohol.

7.1.2 Hydrochloric acid.

7.1.3 Perchloric acid

7.1.4 Ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$].

7.1.5 Ammonium hydroxide.

7.1.6 Sodium metabisulphite ($Na_2S_2O_5$).

7.1.7 Sodium sulphite (Na_2SO_3).

7.1.8 1-amino-2-naphthol-4-sulphonic acid.

7.1.9 Sodium acetate.

7.1.10 Trichloroacetic acid.

7.1.11 Diethyl ether.

7.1.12 Ethanol, 95 % (*V*/*V*).

7.1.13 Cellulose powder, for thin layer chromatography.

7.1.14 Soluble starch.

7.1.15 Reference mixture.

Dissolve in 100 ml of water.

— 50 mg of sodium dihydrogen phosphate (NaH_2PO_4),

— 50 mg of tetrasodium diphosphate ($Na_4P_2O_7$),

— 50 mg of pentasodium triphosphate ($Na_5P_3O_{10}$), and

— 50 mg of sodium hexametaphosphate ($Na_6P_6O_{18}$).

The reference mixture is stable at 4 °C for at least 4 weeks.

7.1.16 Developing solvent.

Mix 120 ml of isopropyl alcohol (7.1.1), 60 ml of a 135 g/l solution of trichloroacetic acid (7.1.10), and 0,6 ml of ammonium hydroxide (7.1.5).

Keep the solvent in a tightly closed bottle.

7.1.17 Spray reagent I.

Add slowly 85 ml of a 40 g/l solution of ammonium molybdate tetrahydrate (7.1.4) to a mixture of 10 ml of a 1 mol/l solution of hydrochloric acid (7.1.2) and 5 ml of a 60 % solution of perchloric acid (7.1.3).

Prepare the reagent on the day of use.

7.1.18 Spray reagent II.

Dissolve 0,5 g of 1-amino-2-naphthol-4-sulphonic acid (7.1.8) in a mixture of 195 ml of a 150 g/l solution of sodium metabisulphite (7.1.6) and 5 ml of a 200 g/l solution of sodium sulphite (7.1.7). Dissolve 40 g of sodium acetate (7.1.9) in this mixture.

Store the reagent in a tightly closed brown bottle in the refrigerator. Discard the solution after 1 week.

7.2 Apparatus

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

7.2.1 Glass plates, thoroughly degreased, 10 cm×20 cm.

7.2.2 Spreading device, for preparing layers of 0,25 mm thickness.

7.2.3 Laboratory mixer.

7.2.4 Desiccator.

7.2.5 Mechanical meat mincer, cutting mills or knife mills, laboratory size, used for homogenizing samples.

7.2.6 Fluted filter paper, of diameter 15 cm.

7.2.7 Micro-pipette, 1 µl, or **micro-syringe** with micrometre screw and bent glass tip.

7.2.8 Paper-lined glass tank, of appropriate dimensions, with tightly fitting lid, for development of thin-layer chromatograms.

7.2.9 Hair-dryer, capable of providing room temperature or a warm air stream.

7.2.10 Sprayer.

7.2.11 Oven, capable of being controlled at 60 °C to 100 °C.

7.2.12 Cellulose plates, to analyse polar substances including the analysis of phosphates, 10 cm x 20 cm.

8 Procedure

8.1 Preparation of thin-layer plates

Dissolve 0,3 g of starch (7.1.14) in 90 ml of boiling water and allow to cool. Then add 15 g of cellulose powder (7.1.13) and homogenize in the laboratory mixer (7.2.3) for 1 min.

Apply this slurry onto glass plates (7.2.1) with the spreading device (7.2.2) adjusted to obtain a layer of 0,25 mm.

Air-dry the plates undisturbed for 60 min at room temperature and heat them finally for 10 min at 100 °C.

Store the plates in the desiccator (7.2.4).

Alternatively, ready-to-use cellulose plates may be used (see 7.2.12).

8.2 Preparation of serum

8.2.1 Macerate 50 g of the test sample (6) with 50 ml of water in a beaker by means of a spatula or a flattened stirring rod until a homogeneous mass is obtained, but taking no more than 5 min.

8.2.2 Add 10 g of the trichloroacetic acid (7.1.10) and again mix thoroughly.

8.2.3 Immediately place in a refrigerator for 1 h and then collect the separated serum by decanting through the fluted filter paper (7.2.6).

8.2.4 If the filtrate is turbid, shake once with an equal volume of the diethyl ether (7.1.11). Remove the ether layer with a small pipette and add an equal volume of the ethanol (7.1.12) to the aqueous phase. Shake for 1 min. Allow the mixture to stand for a few minutes and filter through a fluted filter paper (7.2.6).

The serum is stable at 4 °C for 24 h.

8.3 Chromatographic separation

8.3.1 Pour developing solvent (7.1.16) into the developing tank (7.2.8) to a depth of 5 mm to 10 mm and close the tank with its lid. Allow to stand for at least 30 min at ambient temperature, protected from sunlight and draught.

8.3.2 Apply 3 µl of the serum, or 6 µl if the clearing procedure of 8.2.4 was carried out, to the cellulose layer (8.1) on a pencil line drawn at about 1 cm from the bottom. Keep the spots small by applying 1 µl at a time.

Use a warm air stream from the hair-dryer (7.2.9) for drying. After applying the serum, allow the plate to dry at room temperature, until the spots are no longer visible.

Hot air should be avoided because of the danger of hydrolysis of phosphates.

8.3.3 In the same way, apply 3 µl of the reference mixture (7.1.16) to the plate at a distance of 1 cm to 1,5 cm from the sample spot, but at exactly the same distance from the bottom.

8.3.4 Remove the lid from the tank and quickly but carefully place the cellulose plate in the tank. Replace the lid immediately. Develop the plate at ambient temperature, protected from sunlight and draught.

8.3.5 Continue the development until the solvent front has ascended 6 cm to 10 cm from the pencil line. Remove the plate from the tank and dry for 10 min in the oven (7.2.11) controlled at 60 °C, or alternatively, for 30 min at ambient temperature, or in a stream of cold air.

8.4 Detection of phosphates

8.4.1 Place the plate vertically under a fume hood and spray the plate lightly but uniformly with spray reagent I (7.1.17). Yellow spots appear immediately.

8.4.2 Dry the plate in a stream of warm air from the hair dryer (7.2.9). Subsequently heat in the oven (7.2.11) for 5 min at 100 °C. Remove the plate from the oven.

8.4.3 Allow the plate to cool to room temperature and then replace it under the fume hood. Spray the plate lightly but uniformly with spray reagent II (7.1.18). Blue spots appear immediately.

NOTE Spraying with reagent II is not an absolute necessity. However, the intense blue spots produced by this reagent improve the detection considerably.

8.5 Limit of detection

The limit of detection (LOD) for orthophosphate, diphosphate (pyrophosphate), triphosphate and hexametaphosphate (Graham's salt) is 0,5 g/kg.

8.6 Repeatability and reproducibility

The repeatability and reproducibility of the method was validated by an interlaboratory test (See Annex A).

9 Interpretation

Compare the migration distances of the phosphate spots from the sample with those of the phosphates from the reference mixture.

An orthophosphate spot is always present. If the sample contained condensed phosphates, a diphosphate spot and/or spots of more highly polymerized phosphates are visible.

The R_F values of the phosphates in the reference mixture are:

orthophosphate	from 0,70 to 0,80
diphosphate (pyrophosphate)	from 0,35 to 0,50
triphosphate	from 0,20 to 0,30
hexametaphosphate (Graham's salt)	0,0

Generally, the R_F values of the condensed phosphates in extracts of meat and meat products are somewhat lower.

Note that the R_F values of the above are only for reference. The R_F values may be different in each laboratory. Each laboratory shall determine its own R_F values during the test. Corrections for the differences in R_F values of the phosphates in the sample extract and in the reference mixture can be obtained by placing an extract of the fresh meat sample on the same plate. As fresh meat only contains monophosphates, the percentage correction can be obtained by comparison of the migration distances of this standard spot with the corresponding spot from the reference mixture.

See thin layer chromatograms of reference mixture shown as Figure B.1.

10 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this document, i.e. ISO 5553:2023;

— all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result;

— the test result obtained, 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)

Interlaboratory test

The interlaboratory test of this document was carried out from August 2022 to October 2022.

Eight laboratories participated in 2 parallel tests on 4 samples.

The test method described in this document was adopted here for detection of condensed phosphates in meat and meat products samples.

Four different kinds of meat samples (A pork, B beef, C chicken and D duck) were used during the test. The samples were spiked with different kinds of condensed phosphates, and the concentration levels of positive samples were 0,5 g/kg (LOD), 1,0 g/kg, 2,5 g/kg.

The statistical analysis shows that in above concentration ranges, the test results of condensed phosphates for the 4 samples from participating laboratories are extremely consistent and in line with expectations.