International Standard

ISO 5553

Meat and meat products — Detection of polyphosphates

Viandes et produits à base de viande — Recherche des polyphosphates

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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 5553 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1979.

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

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

No member body expressed disapproval of the document.

Meat and meat products — Detection of polyphosphates

1 Scope

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

2 Field of application

Since polyphosphates are gradually hydrolyzed by enzymes present in the meat or meat product and during heat treatment of the meat or meat product, this International Standard only applies to the detection of added polyphosphates that are still present in the sample at the time of investigation.

3 Reference

ISO 3100, *Meat and meat products — Sampling.*

4 Principle

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

5 Reagents

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

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

5.1 Trichloroacetic acid.

5.2 Diethyl ether.

5.3 Ethanol, 95 % (V/V).

5.4 Cellulose powder, for thin layer chromatography.

5.5 Soluble starch.

5.6 Reference mixture.

Dissolve in 100 ml of water.

— 200 mg of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$),

— 300 mg of tetrasodium diphosphate decahydrate ($Na_4P_2O_7.10H_2O$),

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

— 200 mg of sodium hexametaphosphate ($NaPO_3)_x [x > 10]$.

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

5.7 Developing solvent.

Mix 140 ml of isopropyl alcohol, 40 ml of a 135 g/l solution of trichloroacetic acid, and 0,6 ml of ammonium hydroxide, $\varrho_{20} = 0,90$ g/ml, about 25 % (m/m) solution.

Keep the solvent in a tightly closed bottle.

5.8 Spray reagent I.

Mix equal volumes of a 75 g/l solution of ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$] and concentrated nitric acid ($\varrho_{20} = 1,40$ g/ml) and dissolve 10 g of tartaric acid in 100 ml of this mixture.

Prepare the reagent on the day of use.

5.9 Spray reagent II.

Dissolve 0,5 g of 1-amino-2-naphthol-4-sulphonic acid in a mixture of 195 ml of a 150 g/l solution of sodium disulphite (sodium metabisulphite; $Na_2S_2O_5$) and 5 ml of a 200 g/l solution of sodium sulphite (Na_2SO_3). Dissolve 40 g of sodium acetate trihydrate ($NaOOCCH_3.3H_2O$) in this mixture.

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

6 Apparatus

Usual laboratory equipment not otherwise specified and the following items :

6.1 **Glass plates**, thoroughly degreased, 10 cm × 20 cm.

6.2 **Spreading device**, for preparing layers of 0,25 mm thickness. If such a device is not available, ready-to-use thin-layer plates with layer thicknesses of 0,25 mm can be used provided that starch is used as the binder. Plates containing gypsum (calcium sulphate) are not suitable.

6.3 **Laboratory mixer.**

6.4 **Desiccator.**

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

6.6 **Fluted filter paper**, of diameter 15 cm.

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

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

6.9 **Hair-dryer**, capable of providing either an air stream at room temperature or a warm air stream.

6.10 **Sprayer.**

6.11 **Oven**, capable of being controlled at 60 °C.

7 Sample

7.1 Proceed from a laboratory sample of at least 200 g. See ISO 3100.

7.2 Prepare the test sample on the day of its receipt in the laboratory.

8 Procedure

8.1 Preparation of thin-layer plates

Dissolve 0,3 g of starch (5.5) in 90 ml of boiling water. Cool, add 15 g of cellulose powder (5.4) and homogenize in the laboratory mixer (6.3) for 1 min.

Apply this slurry onto glass plates (6.1) with the spreading device (6.2) 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 (6.4).

Alternatively, ready-to-use thin-layer plates may be used (see 6.2).

8.2 Preparation of the test sample

Homogenize the sample by passing it at least twice through the meat mincer (6.5) and by mixing. 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.

8.3 Preparation of serum

8.3.1 Macerate 50 g of the test sample (8.2) with 15 ml of water at 40 to 60 °C 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.

8.3.2 Add 10 g of the trichloroacetic acid (5.1) and again mix thoroughly.

8.3.3 Immediately place in a refrigerator for 1 h and then collect the separated serum by decanting through the fluted filter paper (6.6).

8.3.4 If the filtrate is turbid, shake once with an equal volume of the diethyl ether (5.2). Remove the ether layer with a small pipette and add an equal volume of the ethanol (5.3) to the aqueous phase. Shake for 1 min. Allow the mixture to stand for a few minutes and filter through a fluted filter paper (6.6).

8.4 Chromatographic separation

8.4.1 Pour developing solvent (5.7) into the developing tank (6.8) to a depth of 5 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 draughts.

8.4.2 Apply 3 μl of the serum, or 6 μl if the clearing procedure of 8.3.4 was carried out, to the cellulose layer (8.1) on a pencil line drawn at about 2 cm from the bottom. Keep the spots small by applying 1 μl at a time.

Use a warm air stream from the hair-dryer (6.9) for drying.

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

8.4.3 In the same way, apply 3 μl of the reference mixture (5.6) to the plate at a distance of 1 to 1,5 cm from the sample spot, but at exactly the same distance from the bottom.

8.4.4 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 draughts.

8.4.5 Continue the development until the solvent front has ascended approximately 10 cm from the pencil line. Remove the plate from the tank and dry for 10 min in the oven (6.11) controlled at 60 °C, or alternatively, for 30 min at ambient temperature, or in a stream of cold air.

8.5 Detection of phosphates

8.5.1 Place the plate vertically under a fume hood and spray the plate lightly but uniformly with spray reagent I (5.8).

Yellow spots appear immediately.

8.5.2 Dry the plate in a stream of warm air from the hair dryer (6.9). Subsequently heat in an oven for at least 1 h at 100 °C to remove the last traces of nitric acid. Remove the plate from the oven and verify the absence of the pungent smell of nitric acid.

8.5.3 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 (5.9).

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.

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 0,80 to 0,90
diphosphate (pyrophosphate)	from 0,50 to 0,60
triphosphate	from 0,25 to 0,35
hexametapolyphosphate (Graham's salt)	0,0

Generally the R_F values of the polyphosphates in extracts of meat and meat products are somewhat lower.

NOTE — 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.

10 Test report

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

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

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