DETECTION OF POLYPHOSPHATES

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INTRODUCTION

Polyphosphates (food grade) are commonly used in the production of fish jelly products. The addition of polyphosphates helps to smoothen the ground fish paste and increase the gel strength of the final fish jelly products. The commercial polyphosphate is a 1:1 mixture of sodium pyrophosphate and sodium tripolyphosphate (sodium triphosphate).

The principle of detection involves extracting the polyphosphates present in the sample with trichloroacetic acid, separating the phosphates by thin layer chromatography (TLC) and finally detecting the phosphates by spraying with colour reagent.

This method is also applicable to meat and meat products.

PREPARATION OF SAMPLE SOLUTION

1. Mix well 50 g minced sample with 15 ml warm water using a spatula.
2. Add 10 g trichloroacetic acid and mix.
3. Store in the refrigerator for one hour to allow separation.
4. Filter the separated solution.
5. Collect the clear solution for chromatographic separation.

N.B. 1. If the filtrate is turbid, add an equal volume of diethylether and shake. Remove the ether layer with small pipette and add an equal volume of 95% ethanol to the water phase. Shake for a minute. Allow the mixture to stand for a few minutes before filter.

2. Use the sample solution on the day of preparation. Store it chilled if the chromatography analysis cannot be done immediately.

REAGENTS

a) Preparation of TLC plates

1. Weigh 15 g of cellulose powder, Whatman thin layer chromedia CC41, into a beaker.
2. Add 30 ml distilled water and mix well with glass rod.

3. Apply this slurry onto glass plates (20 x 20 cm) with the spreading device to obtain a layer of 0.25 mm in thickness.

4. Air-dry the plates undisturbed for 60 min at room temperature.

5. Heat them finally for 10 min at 100°C.

6. Store the plates in a desiccator.

b) Preparation of developing solvent of TLC

Isopropyl alcohol, 140 ml.
Trichloroacetic acid (TCA), 40 ml of 13.5% solution.
Ammonia, 0.6 ml (SG 0.91).

Mix these solutions. If the solvent is not to be used on the same day of preparation, keep it in a tightly closed bottle.

c) Preparation of spray reagents

Spray reagent I

7.5% ammonium molybdate solution
Concentrated nitric acid (analytical grade)

Mix equal volumes (1:1). Prepare the reagent on the day of use.

Spray reagent II

195 ml of 15% sodium metabisulphite solution
5 ml of 20% sodium sulphite
0.5 g 1-amino-2-naphthol-4-sulphonic acid

Mix and store the reagent in a closed brown bottle in the refrigerator.

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

d) Preparation of polyphosphate standards

Sodium dihydrogen orthophosphate monohydrate, $NaH_2PO_4.H_2O$
Tetrasodium diphosphate decahydrate, (sodium pyrophosphate) $Na_4P_2O_7.10H_2O$
Pentasodium triphosphate, (sodium tripolyphosphate) $Na_5P_3O_{10}$
Sodium hexametaphosphate $(NaPO_3)_6$, Gramham's salt

Dissolve 200 to 300 mg of each of the standards in 100 ml of distilled water. These standard solutions can be kept at 40°C for 4 weeks.

APPARATUS

1. Oven (Temp 30 - 200°C)
2. Glass chamber tank with cover
3. TLC plates (20 x 20 cm)
4. Glass tips

CHROMATOGRAPHIC SEPARATION OF POLYPHOSPHATES

1. Fill a paper-lined developing chamber with the developing solvent up to a layer of 0.5 to 1 cm over the bottom. Close the chamber immediately with a tightly fitting lid.

2. Allow to stand for at least 30 min at ambient temperature in order to saturate the chamber atmosphere with the vapour of the developing solvent. This system should be protected from sunlight and draught.

3. Apply 5 µl of the sample solution on to the TLC plate at about 2 cm from the bottom end of the plate. Keep the spots small by applying 1 µl at a time. Use a cold air stream for drying.

   N.B. Hot air should be avoided because of danger of hydrolysis of polyphosphates.

4. In the same way, apply 5 µl of the standard solutions on the plate at an interval of 1.5 - 2 cm, but at exactly the same distance from the bottom end of the plate.

5. Remove the lid from the chamber and quickly but carefully place and dip the spotted plate in the developing solvent in the chamber. Replace the lid immediately.

6. Develop the plate until the solvent front has ascended about 10 cm.

7. Remove the developed plate from the chamber, mark the position of the solvent front with pencil, and allow to dry at ambient temperature for 30 min or, alternatively, in a stream of air.

8. Place the plate under a fume hood and spray the plate lightly but uniformly with spray reagent I.

9. Air-dry the plate under a fume hood. Subsequently heat for 30 min at 100°C in the oven in order to remove the last traces of nitric acid and decompose polyphosphate.

10. Remove the plate from the oven and verify the absence of the pungent smell of nitric acid. Yellow spots will slightly appear in the presence of phosphate.

11. 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. Blue spots will appear immediately on phosphate areas.

12. Measure the migrating distance from the spotting position to the center of the phosphate spot (A) and also to the solvent front (B).

13. Calculate the ratio A/B (Rf value).

INTERPRETATION OF RESULTS

Compare the migrating distance of the phosphate spots from the sample with those of the standard solution. The Rf values of some phosphates are:

Orthophosphate	0.80 - 0.90
Diphosphate (Pyrophosphate)	0.40 - 0.45
Triphosphate	0.20 - 0.23
Hexametapolyphosphate	0.00

These values are changeable according to developing conditions. It is advisable to analyse sample solution together with the standard solution.

REFERENCE

International Standard Orgn. ISO/TC 34/Sc 6/WG 2. The Netherlands.