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Title: Determination of Phosphate		
Revision: 01	Replaces: PHS1, 1993	Effective: 08/10/2009

Contents

A. INTRODUCTION

1. Theory

A partially dried comminuted meat sample is digested by heating with a mixture of nitric and hydrochloric acids. The resultant solution is diluted with distilled water and filtered. An aliquot of filtrate is further diluted with distilled water. The diluted filtrate is heated with a known amount of quimociac reagent to form precipitates of quinolinium phosphomolybdate. The precipitates are filtered, washed with distilled water, dried and quantitated by gravimetric determination.

2. Applicability

This method is applicable to the determination of Phosphate in processed meat products at level ≥ 0.10%.

B. EQUIPMENT

Note: Equivalent apparatus may be substituted.

1. Apparatus

a. Glass fiber filter paper - 12.5 cm circles, Cat. No. 1005 070, Whatman

b. Gooch Crucibles - 40 mL capacity, Cat. No. 60151, CoorsTek

Note: Preparation of the crucible: Place Gooch crucible containing glass fiber filter disc in vacuum flask. Center disc and wash with approximately 50 mL H_2O. Dry the crucible at 250 ± 2 ºC for 30 min in a forced draft oven. Cool in desiccator.

c. Muffle furnace - Cat. No. 10-549-110C, Fisher Scientific

d. Oven - Forced Draft oven, Cat. No. 13-258-11C, Fisher Scientific

e. Desiccator - Cat. No. 08-632. Fisher Scientific

f. Ashing dish - 45 mL flat bottom, Cat. No. 13180-45, Corning Life Sciences

g. Steam bath - manufactured in-house

h. Kohlrausch or sugar flasks – 200mL, Cat. No. 10-462-852, Fisher Scientific

i. Beakers - 250 mL

j. Graduated Cylinders – 25, 50, and 1000 mL glass or plastic

k. Pipettes – 250 µL electronic or manual

l. Hot plate - Cat. No. 11-499D, Fisher Scientific

m. Filter papers - 240 mm Folded Filters, Catalog No. 10314720, Whatman

n. Analytical balance, capable of measuring 0.1 mg

o. Top Loading balance - Cat. No. 11378-834, VWR International

p. Volumetric flasks - 100 mL, class A, Pyrex

q. Erlenmeyer Flasks - 500 mL

r. Watch Glass - Cat. No. 9985-90, Corning

s. Tippet, 5 mL - Cat. No. 53481-348, VWR International.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted.

1. Reagents

a. Sodium molybdate dihydrate - Cat. No. 3764-01, J.T. Baker

b. Citric acid monohydrate - Cat. No. 0627-12, Mallinckrodt Chemicals

c. Synthetic Quinoline - Cat. No. Q35-500, Fisher Scientific

d. Acetone - Cat. No. LP010-4, Honeywell Burdick & Jackson

e. Concentrated HNO_3 - Cat. No. 9601-34, J.T. Baker

f. Concentrated HCl - Cat. No. 9530-33, J.T. Baker

g. Water - Distilled or deionized.

2. Solutions

a. Dilute nitric acid (1:4):

Add 200 mL of concentrated HNO_3 to 800 mL of H_2O, mix well.

b. Quimociac reagent :

i. Dissolve 70 g sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$) in 150 mL H_2O.

ii. Dissolve 60 g citric acid monohydrate in a mixture of 85 mL of concentrated HNO_3 and 150 mL H_2O and cool.

iii. Gradually add the molybdate solution to the citric-nitric acid solution while stirring.

iv. Dissolve 5 mL synthetic quinoline, with stirring, in a mixture of 35 mL of concentrated HNO_3 and 100 mL H_2O.

v. Gradually add this solution to the molybdic-nitric acid solution, mix well and let stand for 24 hours.

vi. Filter, add 280 mL of acetone, dilute to 1 L with H_2O, and mix. Store in either a noncolored polyethylene bottle or a dark brown glass bottle.

D. STANDARDS

Note: Equivalent standards and concentration may be substituted.

1. Source – Sodium Triphosphate ($Na_5O_{10}P_3$), Cat. No. 72061, Sigma Aldrich

2. Preparation – Fortification solution (0.04 g/mL)

   Weigh 4 g of neat sodium triphosphate into 100 mL volumetric flask and bring to volume with distilled water.

3. Storage and Stability– Standard solution is stable for up to 6 months refrigerated.

E. SAMPLE PREPARATION

Process samples until homogeneous.

F. ANALYTICAL PROCEDURE

1. Wet Ashing

   a. Weigh 2.0 ± 0.1g sample into a 200 mL sugar flask, using filter paper to wrap the sample to prevent the meat from adhering to the neck of the flask.

      i. Controls

      Prepare positive and negative tissue controls at this time, as well as the reagent blank. Roll an empty filter paper and place in flask for the reagent blank, and then weigh two portions of blank tissue into separate flasks. Fortify one with the fortification solution to obtain 0.1%Phosphate.

   b. Add 5 mL of concentrated HCl and 30 mL of concentrated HNO_3 to the flask.

   c. Place flask on a hot plate (in a hood) and digest the sample until approximately 15 mL of solution remains.

      Note: Caution! Do not let it go to dryness.

   d. Cool flask in hood; bring to volume with distilled water. Use the bottom of the fat layer as the meniscus. Mix thoroughly.

   e. Filter a portion of the solution, through a filter paper and pipet a 25 mL aliquot

into a 500 mL flask. Add 75 mL of distilled H_2O.

Note: Continue at F.3.

2. Dry Ashing (Optional)

Note: An alternative dry ashing procedure may be used instead.

a. Weigh 2.5± 0.1 g (no more than 780 mg of QPM) of sample into an ashing dish and dry for 30 min at 125 ^oC in a forced draft oven.

b. Ash at 550 ^oC in muffle furnace until white ash is obtained.

c. Cool, and add 25 mL of dilute nitric acid, and heat on steam bath for 30 min.

d. Filter into a 500 mL beaker. Wash dish and paper with distilled water so that total volume in the beaker is approximately 100 mL. Continue with steps F.3.b-d.

3. Determination

a. Add 50 mL of quimociac reagent, cover with a watch glass, and boil until a clear solution and visible precipitation forms. (Note: it often takes longer than a minute to complete the reaction and obtain a clear solution). Do not use an open flame.

b. Cool to room temperature (swirl flask several times during cooling). Transfer the precipitate to the pre-weighed prepared crucible and wash 5 times with 25 mL portion of distilled H_2O. Using a vacuum flask, allow each portion to drain through before adding the next portion.

c. Dry crucible and contents for 30 min at 250 $^oC \pm 2\ ^oC$, cool in a desiccator, and weigh.

G. CALCULATIONS

1. Procedure

$$\text{Phosphorus Content} = \frac{[(100)(A - B)(0.014)]}{C} - 0.0106\ (\%\ \text{meat protein})$$

Where:

A = Weight of sample precipitate

B = Weight of blank precipitate

C = Sample weight

0.014 = Gravimetric factor derived from:

Atomic weight of phosphorus = 30.97

Molecular weight of QPM = 2212.71 = $(C_9H_7N)_3H_3PO_4 \cdot 12MoO_3$

$$\frac{P}{QPM} = \frac{30.97}{2212.71} = 0.014$$

0.0106 = Factor to correct for the natural phosphorus content of meat protein.

Phosphate content = (Phosphorus Content) (F)

$$F = \frac{\text{Anhydrous molecular weight of desired phosphate}}{(X)\ (\text{Atomic Weight of phosphorus})}$$

Where X = number of atoms of phosphorus in one molecule of the phosphate.

The following table lists phosphates and their corresponding factors

Sodium Phosphates	Factor (F)	Potassium Phosphates	Factor (F)
Na_2HPO_4	4.58	K_2HPO_4	5.61
$(NaPO_3)_6$	3.29	----------	----------
$Na_5P_3O_{10}$	3.96	$K_5P_3O_{10}$	4.82
$Na_4P_2O_7$	4.29	$K_4P_2O_7$	5.32
NaH_2PO_4	3.87	KH_2PO_4	4.39
$Na_2H_2P_2O_7$	3.58	---------	-------

In the event that the sodium phosphate used is not known, use the 3.96 factor to calculate added phosphate.

In the event that the potassium phosphate used is not known, use the 4.82 factor to calculate added phosphate.

In the event that a mixture of phosphates is used, use the factor for the phosphate present that will result in the highest value.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment – Protective clothing, eye protection, gloves and hood where applicable.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
HCl	Will cause severe burns and severe skin, eye, and respiratory irritation.	Prepare solutions in a fume hood. Avoid contact with metal or oxidizing materials
HNO_3	Will cause severe burns to all body tissue. May be fatal if swallowed or inhaled. Will react with water or steam to produce heat and toxic and corrosive fumes.	Prepare solutions in a fume hood. Store out of direct sunlight. Regulate contact with heat, water, and incompatible materials.
Quinoline	Will cause burns and harmful if it comes in contact with the skin. Eyes and respiratory irritant. Risk of serious damage to eyes. Limited evidence of carcinogenic effect.	Use only in fume hood.
Sodium molybdate dihydrate	Respiratory, skin, and eye irritant.	
Citric Acid	Respiratory, skin, and eye irritant	

Acetone	Respiratory tract and eye irritant. May be harmful if absorbed by the skin.	Use only in fume hood.

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
HCl and HNO_3	See Above	Neutralize and flush down the sink
Quimociac	See above	Neutralize and collect waste in tightly sealed container and store away from non-compatibles for disposal in accordance with local, state and federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range*	*Acceptable Recovery (%)*	*Acceptable Reproducibility (%CV)*	*Acceptable Repeatability (%CV)*
Phosphate	≥ 0.10%	70 - 110 %	<20	<15

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	Quimociac Solution	Must be filtered before each use
b.	Sample size	Meat – 2.5 g ± 0.1 g for dry ash; 2.0 g ± 0.1 g for wet ash. Cures, spices, etc. – smaller aliquots or greater dilutions are required than for meat. Sample size for any sample must not exceed 25 mg P_2O_5 or a quinolinium phosphomolybdate (QPM) precipitate weight of about 0.78 g.

c.	Wet Ash	Screening method only. Do not allow to go to dryness. Use hood.
d.	Muffle furnace temperature	550 ^{o}C (Do not exceed 600 ^{o}C)
e.	Final oven temperature and time	250 ^{o}C ± 2 ^{o}C for 30 min after oven recovers to 250 ^{o}C

3. Readiness To Perform

   a. Familiarization

      i. Phase I: Not applicable.

      ii. Phase II: On two separate days, analyze a blank and 2 fortified samples in duplicate from 0.1 up to 0.6%Phosphate.

      iii. Phase III: Check samples for analyst accreditation.

         (a) 6 total blind samples must be analyzed at least 1 must be blank and the remaining must be fortified between 0.1 and 0.6%Phosphate.

         (b) Report analytical findings to Quality Assurance Manager (QAM) or Supervisor.

         (c) Authorization from QAM and supervisor is required to commence official analysis.

   b. Acceptability criteria.

      Refer to I. 1.

4. Intralaboratory Check Samples

   a. System, minimum contents.

      i. Frequency: Once per week per analyst when samples are analyzed.

      ii. Records are to be maintained

   b. Acceptability criteria.

      Refer to I. 1.

      If unacceptable values are obtained, then:

      i. Stop all official analyses by that analyst.

      ii. Take corrective action.

5. Sample Acceptability and Stability

   a. Matrix: Processed meat products

   b. Sample receipt size, minimum: 1lb.

c. Condition upon receipt: Cold and sealed from air.

d. Sample storage:

i. Time: 1 week

ii. Condition: 4 ^{o}C refrigerated or kept indefinitely if frozen.

6. Sample Set

a. Tissue Blank.

b. Recovery.

c. Reagent Blank.

d. Samples

7. Sensitivity

a. Minimum proficiency level (MPL): 0.10 % Phosphate

J. WORKSHEET

{Reserved}

K. APPENDIX

1. Reference

Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Edition, 969.31B.

L. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.