PHOSPHORUS, TOTAL AND BOUND(Colorimetric)

PRINCIPLE

The sample is ignited in the presence of a fixative to destroy organic matter and convert phosphorus to inorganic phosphates which are not volatilized during ignition. Residual phosphates are taken up in acid, hydrolyzed to orthophosphate and determined spectrophotometrically as a reduced phosphomolybdic acid complex (Note 1). For bound phosphorous the sample is first washed thoroughly with an aqueous solvent to remove soluble phosphorus-containing materials, and then ignited. (Note 2)

SCOPE

The method contains a procedure applicable to the determination of total phosphorus in corn syrups, corn sugars and modified and unmodified starches obtained from the corn wet milling process and also contains a procedure for bound phosphorous applicable to unmodified, modified and derivatized granular starches. With only minor modification, the procedure can be applied to other cold water insoluble products obtained from the wet milling of corn and grain sorghum (Note 3).

SPECIAL APPARATUS

1. VYCOR Dishes: 100 mL capacity- Do not use platinum (Note 4).

2. Muffle Furnace: Equipped with a pyrometer and capable of operating at controlled temperatures up to 650 °C

3. Spectrophotometer: An instrument capable of accurate absorbance measurements at 460 nm and 825 nm and equipped with a red-sensitive detector and matching 1.0 cm cuvettes.

REAGENTS

1. Zinc Aetate Solution, 10%: Dissolve 120.0 g of reagent grade zinc acetate dihydrate [$Zn(C_2H_3O_2)\bullet H_2O$] in 880 mL of purified water. If the solution is hazy, filter through Whatman No. 2V filter paper.

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2. Nitric Acid Solution, 29%: Add 300 mL of concentrated nitric acid (70% HNO_3, sp g 1.42) to 600 mL of purified water and mix.

3. Sulfuric Acid Solution, 26%: Cautiously add 167 mL of reagent grade concentrated sulfuric acid (96% H_2SO_4, sp g 1.84) to 833 of purified water and mix thoroughly.

4. Ammonium Molybdate Solution, 2%: Dissolve 10.6 g of reagent grade ammonium molybdate tetrahydrate [$(NH_4)Mo_7O_{24}\bullet 4H_2O$] in 500 mL of purified water and mix thoroughly.

5. Ammonium Molybdate Solution, 5%: Dissolve 50 g of ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\bullet H_2O$) in 900 mL of warm purified water. Cool to room temperature and dilute to 1 L with purified water.

6. Ascorbic Acid Solution, 5%: Dissolve 5.0 g of ascorbic acid in 100 mL of purified water. Make fresh every 48 hrs.

7. Methanol-Water, 7:3 (V/V): Add 700 mL of reagent grade methanol to 300 mL of purified water and mix.

8. Ammonium Vanadate Solution, 0.25%: Dissolve 2.5 g of ammonium metavanadate ($(NH_4)VO_3$) in 600 mL of boiling purified water. Cool to 60-70 °C and add 20 mL of concentrated nitric acid. Cool to room temperature, transfer quantitatively to a 1 L volumetric flask and dilute to mark with purified water.

9. Standard Phosphorus Solution, 0.1 mg/mL as Phosphorus:

   Dissolve exactly 0.4395 g (Note 5) of reagent grade potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of purified water in a 1 L volumetric flask; dilute to volume with purified water and mix thoroughly.

   Standard Solution: 2 µg Phosphorus per mL:

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Pipet 10.0 mL of the stock solution into a 500 mL volumetric flask; dilute to volume with purified water and mix thoroughly.

PROCEDURE

For total phosphorous:

Standardization: Pipet 5.0, 10.0, 15.0, 20.0 and 25.0 mL of the standard phosphorus solution (respectively 10, 20. 30, 40 and 50 µg of phosphorus) into respective 50 mL volumetric flasks (Note 6); reserve another flask for a blank. To each flask add, in order, and mix after each addition, 10 mL of 26% sulfuric acid solution and 5 mL of 2% ammonium molybdate solution. Add purified water to make a total volume of about 45 mL; add 2 mL of 5% ascorbic acid solution and mix thoroughly. Place the flasks in a boiling water bath for 10 mins. (Note 7). Cold to 25 °C in an ice bath. Dilute to volume with purified water and mix thoroughly. Using the blank as a reference at O absorbance (A), determine A of each standard in a 1 cm cuvette at 825 nm. Plot A against µg of phosphorus per 50 mL.

Analysis: Weigh 2 g to the nearest milligram of the sample or an amount containing not more than 500 µg of phosphorus in a 100 mL VYCOR dish. Add 10 mL of 10% zinc acetate solution; distribute the solution uniformly through the sample, adding purified water if necessary. Evaporate to dryness on a steam bath and char the sample on a hot plate. Ignite in a muffle furnace at 550 °C for 2 hrs. Cool to room temperature and wet all of the residue by cautious addition of 3 mL of 29% nitric acid solution (Note 8). Evaporate to dryness on a steam bath and dehydrate by brief heating on a hot plate. Return the dish to the muffle furnace for 20 mins.

Cool to room temperature; carefully wash down the sides of the dish with 3 mL of 26% of sulfuric acid solution and 5 mL of purified water (Note 9). Heat to incipient boiling and hold at that temperature for 10 mins. (Note 10).

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For high level samples less than 500 µg phosphorus:
With the aid of purified water, quantitatively transfer the contents of the dish to a 100 mL volumetric flask. Cool to room temperature, dilute to volume and mix thoroughly. Transfer 10.0 mL to a 50 mL volumetric flask.

For low level samples less than 50 µg phosphorus:
With the aid of purified water, quantitatively transfer the contents of the dish to a 50 mL volumetric flask. Cool to room temperature.

Add 10 mL of 26% sulfuric acid solution, add 5 mL of 2% ammonium molybdate solution, mixing after each addition. Dilute to abouth 45 mL with purified water and add 2 mL of 5% ascorbic acid solution. Mix, heat, cool and dilute as directed under standardization (Note 11). Using the reagent blank as reference at O A (Note 12), determine A of the samples in 1 cm cuvette at 825 nm. Determine µg of phosphorus in the sample from the standardization curve.

For bound phosphorous:

Standardization: Pipet 5.0, 10.0, 15.0, 20.0 and 25.0 mL of standard phosphorus solution (respectively, 0.5, 1.0, 1.5, 2.0 and 2.5 mg of phosphorus) into respective 100 mL volumetric flasks and use another flask for a blank. To each flask add, *in order*, 10 mL of 29% nitric acid, 10.0 mL of 0.25% ammonium vanadate, and 10 mL of 5% ammonium molybdate, mixing thoroughly after addition of each reagent (Note 13). Dilute to volume with water, mix thoroughly and allow to stand for 10 mins. (Note 14). Using the blank as a reference solution at zero absorbance, determine absorbance of each standard at 460 nm. Plot absorbance vs. mg of phosphorus per 100 mL (Note 15).

Sample Preparation: Place 20 to 25 g of granular starch product (Note 16) in a 250 mL beaker and add 200 mL of 7:3 methanol-water at room temperature. Disperse starch and agitate mechanically for 15 mins. Recover starch by vacuum filtration in a 150 mL medium porosity fritted glass or Büchner funnel and wash the wet cake with 200 mL of 7:3 methanol-water. Reslurry and rewash the wet cake with 7:3 methanol-water.

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Dry the washed starch filter cake in an air oven at a temperature below 50 °C. Grind the sample completely through a laboratory cutting mill or with mortar and pestle to 20 mesh or finer and blend thoroughly. Determine dry substance by a standard procedure.

Low-Phosphorus Samples (0.005% to 0.05% P): Weigh accurately in a VYCOR dish an amount of washed and dried starch sample containing not more than 2.5 mg of phosphorus (Note 4). Recommended sample sizes for low-phosphorus starches and other sample types are approximately as follows:

Phosphorus, % as is	*Sample Wt. (g)*
0.01 and lower	20
0.01 to 0.025	10
0.025 to 0.050	5

High-Phosphorus Samples (0.05% to 2.0% P): Weigh accurately an amount of washed and dried starch sample containing not more than 40 mg of phosphorus (Note 16). Recommended sample sizes for high phosphorus starches and other sample types are listed below, together with recommended residue dilution volumes and aliquot sizes for spectrophotometric analysis.

Phosphorus, % as is	*Sample Wt. (g)*	*Dilution Volume (mL)*	*Aliquot Volume (mL)*
0.05 to 0.10	10	100	25
0.10 to 0.25	10	200	20
0.25 to 0.75	5	250	15
0.75 to 2.00	2	250	15

In all cases, add 10 mL of 10% zinc acetate solution to the VYCOR and distribute the solution uniformly through the sample adding water if necessary. Evaporate to dryness on a steam bath and char the sample on a hot plate. Ignite in a muffle furnace at 550 °C for 2 hrs.

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Cool to room temperature and wet all of the residue by cautious addition of 3 mL of 29% nitric acid (Note 17). Evaporate to dryness on a steam bath and dehydrate by brief heating on a hot plate. Return the dish to the muffle furnace for 20 mins.

Cool to room temperature, wash down the sides of the dish with 10 mL of 29% nitric acid and add 15 mL of purified water. Cover with a watch glass, heat to incipient boiling and hold at that temperature for 10 mins. (Note 19).

For low phosphorus samples: Quantitatively filter through Whatman No. 1 filter paper into a 100 mL volumetric flask. Complete the transfer with the aid of four 10 mL portions of purified water. To each flask, add *in order*, 10 mL of 29% nitric acid, 10.0 mL of 0.25% ammonium vanadate, and 10 mL of 5% ammonium molybdate, mixing thoroughly after addition of each reagent (Note 6).

For high phosphorus samples: Filter into a volumetric flask of a size selected to yield a final phosphorus concentration of 1 to 4 mg/mL P. Complete the transfer with the aid of four 10 mL portions of purified water. Dilute to volume with purified water and mix thoroughly. Transfer an aliquot selected to contain not more than 2.5 mg of phosphorus to a 100 mL volumetric flask and add 50 mL of purified water to another flask to serve as a blank. Add, *in order*, 10 mL of 29% nitric acid, 10 mL of 0.25% ammonium vanadate, and 10 mL of 5% ammonium molybdate, mixing thoroughly after addition of each reagent (Note 13).

In all cases, dilute to volume with purified water, mix thoroughly and allow to stand for 10 mins. (Note 14). Determine absorbance of the sample at 460 nm using the blank as a reference solution at zero absorbance. Read mg of phosphorus in the aliquot from the standardization curve.

CALCULATION

For Total Phosphorous

For low level samples:

$$\text{Phosphorus, } \mu g/g = \frac{(\mu g \text{ Phosphorus From Graph})}{\text{Sample Wt., g}}$$

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For high level samples:

$$\text{Phosphorus, } \mu g/g = \frac{(\mu g \text{ Phosphorus From Graph})(10)}{\text{Sample Wt., g}}$$

For Bound Phosphorous:

Low-Phosphorus Samples:

$$\%\text{ (dry basis) Phosphorus} = \frac{(\text{Phosphorus, mg from Graph})(100)}{(\text{Dry Sample Wt., g})(1000)}$$

High-Phosphorus Samples:

$$\%\text{ (dry basis)} = \frac{(\text{Phosphorus, mg from Graph})(\text{Dilution Volume})(100)}{(\text{Aliquot Volume})(\text{Dry Sample Wt., g})(100)}$$

NOTES AND PRECAUTIONS

1. Reduced phosphomolybdic acid ("moly blue") procedures have long suffered from errors arising from instability and lack of reproducibility. The procedure described here is adapted from that of Fogg and Wilkinson [Analyst *83*, 406 (1958)]; in sharp contrast to other procedures of its type, it shows excellent reproducibility and more than adequate color stability.

2. Chemistry of the chromophore is in dispute; for a discussion of some of the possibilities, see C.Y. Shen and D.R. Dyroff, Determination of Phosphate in Presence of Silicates by Molybdenum Blue Method; *Anal. Chem.*, **1962**, Vol. 34, no. 11, pp. 1367-1370.

3. The procedure as written is not recommended for starches containing less than 40 ppm phosphorus. In this low range, adequate precision can be attained by ten-fold dilution of the phosphorus standard, and use of 10 cm cuvettes for analysis of standards and samples.

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4. Platinum *must not* be used, because it may be damaged by the fixative or by large phosphate residues.

5. Most samples of potassium dihydrogen phosphate assay slightly over 100% KH_2PO_4. Obtain the exact weight of standard to be taken by dividing 0.4395 by the decimal purity factor. (Example: Assay = 100.4% KH_2PO_4; decimal purity factor = 1.004; weight of standard = 0.4377 g).

6. As is common in determination of trace constituents, most errors in this procedure stem from use of contaminated equipment. Invisible traces of detergent may contribute as much phosphorus as is contained in the sample. Dishes used for ignition of high-phosphorus samples will often contain significant quantities (e.g., several micrograms) of residual phosphorus. For this analysis, volumetric flasks and pipets should be cleaned with warm chromic acid (or non-phosphorus containing equivalent solution) and rinsed thoroughly with purified water. VYCOR dishes should be treated in boiling concentrated hydrochloric acid and rinsed thoroughly before us. Ideally, glassware used in this procedure should never come in contact with detergents or high-phosphorus samples.

7. Heating time may be shortened to 5 mins. if bath capacity is such that bath temperature does not fall below 90 °C when samples are inserted. Longer heating times (up to 45 mins.) result in green colors, but do not affect net absorbance at 825 nm.

8. The residue will seldom be carbon-free at this point; treatment with nitric acid and reignition assures complete combustion in a reasonable time. Residue from the evaporation contains hydrates which must be decomposed, by heating as directed, to avoid loss by spattering in the furnace.

9. Adhere closely to quantities of acid specified. Excess acid inhibits color development; low acidity results in complete reduction of the molybdate reagent.

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10. Heating as described is necessary even if the residue dissolves immediately. Any pyrophosphate in the residue must be hydrolyzed to orthophosphate before color development.

11. The method is somewhat sensitive to variation in experimental condition; to avoid risk of serious error, include at least one phosphorus standard together with its reagent blank in each set of samples.

12. Zinc acetate dihydrate has not been observed to contribute apparent phosphorus to the system; however, it is advisable to check an ignited zinc acetate blank against a reagent blank whenever a new lot of zinc acetate dihydrate is used.

13. Reagents *must* be added *in order* as stated in order to avoid interferences from precipitation and side reactions.

14. The color increases very slowly for the first four hours, resulting in a relative error of about 2%. With double beam spectrophotometers, error is negligible if readings are made within 2 hrs. of solution preparation or if samples and standards are read at comparable intervals after preparation.
15. The standardization curve is reproducible and needs be checked only when fresh reagents are prepared.
16. If the sample is soluble in cold water, prepare a 1 to 2% aqueous solution (paste); place in a cellophane tube and dialyze against running purified water for 30-40 hrs. Precipitate the starch by pouring the solution into 4 volumes of acetone (per volume of paste) while stirring. Recover precipitated product by vacuum filtration on a fritted glass or Büchner funnel. Wash filter cake with absolute ethanol. Dry in an air or vacuum oven, grind, blend and determine volatiles before analysis. A dry product can also be recovered from the dialyzed paste by freeze-drying.
17. The residue will seldom be carbon-free at this point; treatment with nitric acid and re-ignition assures complete combustion in a reasonable time. Residue from the evaporation contains hydrates which must be decomposed, by heating as directed, to avoid loss by spattering in the furnace.

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18. Part or all of the phosphorus in the residue is present as pyrophosphate, which will not form the molybdovanadophosphoric acid complex. The specified heating period is necessary to hydrolyze pyrophosphate to the reactive orthophosphate. Since higher phosphates, such as trimetaphosphate or tripolyphosphate, may be present (as in unreacted mixtures of starch with polymeric phosphates), it is advisable to extend the heating time to 30 mins.

METHOD HISTORY

Combined the Phosphorus, Total and Bound (Colorimetric) methods for Corn Starch (Unmodified) (B-47), Corn Starch (Modified) (C-46), Corn Starch (Modified) (C-47), Corn Syrup (E-51) and Corn Sugar (F40) on 11-09-2010.

Corn Starch (Unmodified), Phosphorus, Total and Bound (Colorimetric) (B-47), Date of Acceptance 8-03-1973, Revised 10-08-1996.

Corn Starch (Modified), Phosphorus, Bound (Colorimetric) (C-46), Date of Acceptance 10-16-1969, Revised 10-08-1996.

Corn Starch (Modified), Phosphorus, Total and Bound (Colorimetric) (C-47), Date of Acceptance 6-21-74, Revised 10-08-1996.

Corn Syrup, Phosphorus, (Colorimetric) (E-51), Date of Acceptance 6-21-1974, Revised 10-08-1996.

Corn Sugar, Phosphorus, (Colorimetric) (F-40), Date of Acceptance 6-21-1974, Revised 10-08-1996.