Detection of Phosphorus on Starch by Atomic Absorption and the Graphite Furnace

M. L. HOGEN, Industrial Technologist, Technical Service, Grain Processing Corporation, Muscatine, IA 52761

Cereal Chem. 60(5):403-405

ABSTRACT

A new application for Graphite Furnace Atomic Absorption (GFAA) was developed for the detection of phosphorus added to starch, and for that which occurs naturally in starch. An unmodified cornstarch and a modified corn and potato starch, in both a dry and a pasted state, were analyzed for phosphorus, using GFAA. The phosphorus values obtained by GFAA compare well with amounts known to be added to the starches. The most significant advantage in using GFAA rather than colorimetry is that the starch does not have to be digested with perchloric acid to convert the various phosphate forms to orthophosphate. The starch samples are simply acid-hydrolyzed (4*N* hydrochloric acid), then 0.2% nickel is added, instead of the commonly suggested 1–2% lanthanum, to stabilize the phosphorus during heating. Nickel gives equal sensitivity at a lesser cost, and longer graphite tube life than lanthanum.

The objective of this work was to develop a quick and accurate method to detect phosphorus on starch. Conventionally, starch has been prepared for phosphorus detection by wet-ashing or digestion, to convert various phosphate forms to orthophosphate for subsequent colorimetric analysis (Rowe 1973). The most common procedures employ a combination of strong oxidizing agents, such as nitric and perchloric acid, or sulfuric acid plus a mercury catalyst (AOAC 1980, Hambleton 1977). A time-consuming process, orthophosphate conversion during wet-digestion takes 60–90 min. Also, perchloric acid fuming in conjunction with an organic material can be very hazardous unless precautions are taken (ACS 1979). It was desirable to locate an alternate tool for analysis that would enable the starch sample to be simply acid-hydrolyzed and the phosphorus directly detected without subsequent conversion to the orthophosphate form (Simpson and Blay 1966). Over the past 10 years, significant progress has been made in the detection of phosphorus, through the use of Graphite Furnace Atomic Absorption (GFAA).

The determination of phosphorus by GFAA appears to have evolved from the work done by L'Vov (1970), who used an electrically heated graphite cell at the primary resonance lines of 178 nm. The focus then shifted to the use of Flame Atomic Absorption (FAA) at the primary resonance lines. Of course, because of atmospheric absorption, special instrument modifications were needed to operate at this wavelength (Kirkbright and Marshall 1973, Manning and Slavin 1969).

Manning and Slavin (1969) first reported the use of a secondary resonance line doublet at the wavelengths of 213.55 and 213.62 nm, but poor sensitivity was noted when a hollow cathode tube was used as the source. An Electrodeless Discharge Lamp (EDL) was found to greatly enhance the sensitivity for phosphorus detection over the existing hallow cathode tube (Barnett et al 1976). Thus, three main events had finally set the stage for the detection of phosphorus through the use of GFAA: brighter and more stable sources (EDLs); the use of the doublet at 213.55/213.62 nm; and improvement in graphite furnaces. By combining these new features, various researchers (Ediger 1976, Ediger et al 1978, Hobbins 1982, Routh 1979) have optimized the detection of phosphorus by GFAA. When the most sophisticated instrumentation currently available are used, detection limits in the range of 1.5–4 ng P are not uncommon in clean solutions.

Matrix modification has become very important in GFAA analysis (Ediger 1975). During the ashing cycle, various phosphates and phosphides become volatile unless a stabilizer is added. Generally, lanthanum at an addition level of 1–2% has been suggested (Ediger et al 1978, Routh 1979). Hobbins (1982) recently proposed that nickel would stabilize phosphorus during ashing, just as effectively as lanthanum. We tested both nickel and lanthanum, and found that 0.2% nickel was comparable to 1.0% lanthanum in sensitivity. All samples analyzed in this study were stabilized with nickel. This study gives two reasons why GFAA is highly efficient in the detection of various phosphate forms on starch: a lengthy, potentially hazardous wet digestion was eliminated, and no cation/anion interferences were encountered.

MATERIALS AND METHODS

Apparatus

A Perkin-Elmer 460 atomic absorption spectrophotometer equipped with a Perkin-Elmer HGA-2200 flameless atomizer and phosphorus specific EDL was used for all instrument characterization and sample analysis. The GFAA signals were displayed on a Fisher Recordall 5000 strip-chart recorder. The 10- or 20-μl sample aliquots were injected manually, using an Eppendorf pipet with disposable plastic tips. The pyrolytically coated graphite tubes were also obtained from Perkin-Elmer Corporation (Manning and Ediger 1976).

Reagents

The corn and potato starches, used without further purification, were obtained from the following corn refiners: unmodified cornstarch (B200), Grain Processing Corporation; modified cornstarch (Cato 8), National Starch & Chemical Corporation; and modified potato starch (STA-LOK 400), A. E. Staley Manufacturing Company.

All other chemicals were reagent grade and were obtained from Fisher Scientific. The water used for all solutions had been purified by ion exchange. Phosphorus standards used for calibration were prepared from a 1,000 μg/ml stock phosphorus solution made from monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$).

The solution used to hydrolyze the starch was simply 4*N* hydrochloric acid. Nickel, as a stabilizer for the phosphorus during ashing, was added in the nitrate form. A stock solution was prepared containing 10,000 μg/ml nickel in approximately 0.6*M* nitric acid.

Procedure

Before sample preparation, all glassware was rinsed with alcoholic KOH and distilled H_2O to prevent phosphorus contamination. Starch samples were prepared for analysis by weighing 0.5–3 g of granular material or 1–10 g of paste into 125-ml Erlenmeyer flasks. Samples with added phosphorus were spiked with 0.15% phosphorus on dry solid starch, using monobasic sodium phosphate. Next, 25 ml of 4*N* HCl was added to each flask, and the contents boiled gently on a hot plate for 7–10 min. The brown, hydrolyzed starch solutions were allowed to cool to near room temperature. Each sample was quantitatively transferred to 50-ml volumetric flasks containing 10 ml of 10,000 μg/ml Ni stock solution. The samples were diluted to volume with water (purified by distillation followed by ion exchange) and mixed thoroughly. Finally, the samples were filtered through Whatman no. 1 filter

paper, and the filtrate injected into the furnace for phosphorus detection. This filtering step greatly improved both pipetting accuracy and precision.

Phosphorus standards (0, 2, 10, 20, 40, 60, 80, and 100 μg/ml P) were also prepared by the above procedure, although varying amounts of the monobasic sodium phosphate stock solution instead of starch were added to 125-ml Erlenmeyer flasks. When stored in linear polyethylene bottles, these standards remained stable for at least two months. A reagent blank was prepared with each set of starch samples to monitor possible reagent contamination.

RESULTS AND DISCUSSION

Considerable instrument characterization was done before the detection of phosphorus on starch. Because many forms of phosphorus become volatile at about 200° C, some element needed to be added for phosphorus stabilization during the charring cycle. Of the many elements, including Ca, Co, Ct, Cu, Fe, Ni, Zn, La, Ce, Ba, Sr, Mg, and Y, that have been examined as stabilizers (Ediger et al 1978, Hobbins 1982), nickel and lanthanum possessed the largest absorbance enhancement. We tested both elements and determined that during GFAA nickel stabilizes phosphorus as efficiently as lanthanum, but in a significantly reduced quantity. Generally, 1–2% lanthanum is suggested for phosphorus detection, using GFAA, but in this work, 0.2% nickel was found to enhance sensitivity as well as or better than lanthanum. A majority of the instrument characterization was performed using 0.1% nickel in clean phosphorus standards. Excellent sensitivity was achieved even at this reduced level of stabilizer in clean solutions. After some preliminary starch analysis, we decided to increase the level of nickel in all makeups to 0.2%, which was still 5–10 times less than the typical lanthanum level.

After the type and amount of stabilizer had been selected, various studies were run that used phosphorus standards to optimize the following experimental instrument variables: source, phosphorus EDL; EDL power, 8 W; wavelength, 213.6 nm; slit width, alternate 0.7 nm; background, D_2 correction; purge gas, argon (gas stop during atomization); drying cycle, 95° C, 50 sec; charring cycle, 1,400° C, 30 sec; atomization cycle, 2,700° C, 2.5 sec (max power); sample volume, 10 μl (also used 20 μl); stabilizer addition, 0.2% Ni (as the nitrate); detection limit, 0.6 μg/ml P; characteristic concentration (sensitivity), 3 μg/ml P; and normal operating range, 10–100 μg/ml P.

Phosphorus standards were then subsequently analyzed, and a calibration curve was plotted. Absorbance was determined by measuring the peak height in centimeters for each sample. A typical calibration curve (Fig. 1) illustrates that the relationship between increasing amounts of phosphorus versus absorbance is nonlinear. This curve shows that the absorbance pattern above 100 μg/ml phosphorus becomes horizontal and should not be used for quantitation.

The characteristic concentration (sensitivity) and detection limit for phosphorus in this work were determined to be 3 μg/ml and 0.6 μg/ml phosphorus, respectively. The normal working range was selected to be 10–100 μg/ml phosphorus, with the instrumentation available. Hobbins (1982), however, in using the newest, most sophisticated GFAA instrumentation on the market, successfully detected as little as 1.5 ng of phosphorus (absolute detection limit).

Because all pipetting was done manually instead of with an auto injector, a reproducibility study was conducted on the 10- and 100 μg/ml phosphorus standards, using a 20-μl Eppendorf pipet.

Ten injections were made on each standard, and the mean, standard deviation, and relative standard deviation were calculated. Results are shown in Table I.

Ediger et al (1978) determined that, for an auto injector, a relative standard deviation of up to 2% is typical for 20 μl of a 10 μg/ml phosphorus standard solution containing 1% lanthanum. Thus, a 5% relative standard deviation for manual pipetting as determined here indicates that auto injection is needed for very precise phosphorus detection using GFAA.

The detection of phosphorus native to starch or added to starch was then undertaken. To broaden the application of this method, both cornstarch and potato starch were analyzed. Because starch modification (derivatization) and pasting (gelatinization) are common practices in the starch industry, these aspects were also examined. The amounts of phosphorus native to corn and potato starch differ significantly. In general, potato starch contains 5–10 times more inherent phosphorus than cornstarch. In this study, an unmodified cornstarch and a modified corn and potato starch were analyzed for phosphorus under unspiked and spiked conditions. To the spiked samples was added 0.15% P (as monobasic sodium phosphate), which corresponds to a typical level for commercially available derivatized starch phosphates. Each starch sample was

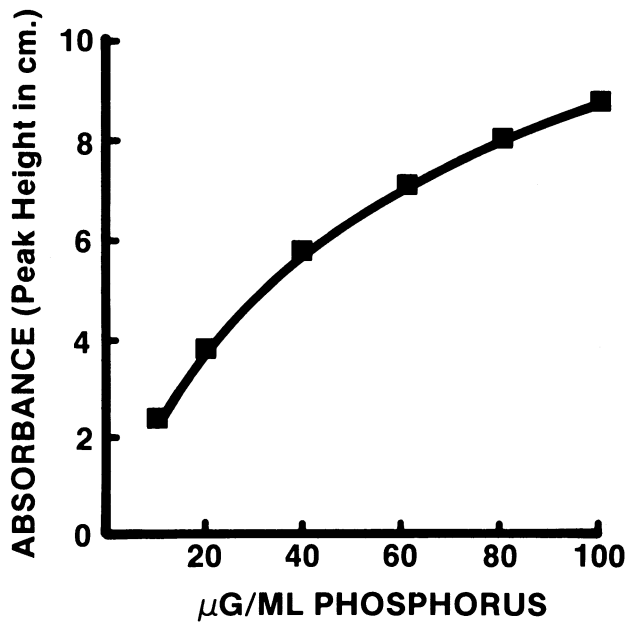

Fig. 1. Typical calibration curve.

TABLE I
Peak Height Reproducibility

Standards (μg/ml P)	Injection Volume (μl)	No. of Injections	Mean (cm)	RSD[a] (%)
10	20	10	2.48	5.22
100	20	10	9.87	3.45

[a] Relative standard deviation.

TABLE II
Analytical Results of the Detection of Phosphorus on Starch Using GFAA

Type of Starch (n = 4)	Known Amount of P Added (%)	Total Percent P Detected (mean)	Standard Deviation (%)	Percent Recovery on Spiked Samples
Unmodified cornstarch				
Granular	0 (unspiked)	0.017	0.001	···
Granular	0.15	0.158	0.009	94
Pasted	0 (unspiked)	0.016	0.003	···
Pasted	0.15	0.156	0.007	93
Modified cornstarch				
Granular	0 (unspiked)	<0.005	···	···
Granular	0.15	0.149	0.006	96-99
Pasted	0 (unspiked)	<0.009	···	···
Pasted	0.15	0.158	0.004	99-105
Modified potato starch				
Granular	0 (unspiked)	0.087	0.005	···
Granular	0.15	0.241	0.006	103
Pasted	0 (unspiked)	0.093	0.003	···
Pasted	0.15	0.264	0.013	114

analyzed in both a granular and a pasted state. Four separate analyses were run on each starch sample, and the mean, standard deviation, and percent recovery calculated. Results of phosphorus detection on starch using GFAA are given in Table II.

The data show that potato starch does contain significantly more naturally occurring phosphorus than does cornstarch. The modified cornstarch possessed an amount of phosphorus smaller than the characteristic concentration, possibly because of derivatization procedures. A typical value for the percent phosphorus of an unmodified cornstarch was 0.017%, on a dry solid starch basis. The calculated percent recovery values for the unmodified and modified cornstarch samples were encouraging, ranging from 93 to 105%. As anticipated before the study began, there was very little difference in the percent phosphorus detected on dry and on pasted starch. The recovery of spiked phosphorus on potato starch was 103% for dry and 114% for pasted starch. A recovery level of 114% was higher than anticipated. This may have resulted from the use of a worn graphite tube, which caused a loss of sensitivity during the calibration of standards.

ACKNOWLEDGMENT

The author would like to thank W. B. Hobbins of Varian Instruments for many helpful discussions during the course of this work.

LITERATURE CITED

AMERICAN CHEMICAL SOCIETY. 1979. Safety in Academic Chemistry Laboratories. 3rd ed. The Society, Washington, DC.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1980. Official Methods of Analysis. 13th ed. The Association, Washington, DC.

BARNETT, W. B., VOLLMER, J. W., and DeNUZZO, S. M. 1976. The application of electrodeless discharge lamps in atomic absorption. At. Absorp. Newsl. 15:33.

EDIGER, R. D. 1975. Atomic absorption analysis with the graphite furnace using matrix modification. At. Absorp. Newsl. 14:127.

EDIGER, R. D. 1976. Standard conditions for the determination of phosphorus with the HGA graphite furnace. At. Absorp. Newsl. 15:145.

EDIGER, R. D., KNOTT, A. R., PETERSON, G. E., and BEATY, R. D. 1978. The determination of phosphorus by atomic absorption using the graphite furnace. At. Absorp. Newsl. 17:28.

HAMBLETON, L. G. 1977. Semiautomated method for simultaneous determination of phosphorus, calcium, and crude protein in animal feeds. J. Assoc. Off. Anal. Chem. 60:846.

HOBBINS, W. B. 1982. Direct determination of phosphorus in aqueous matrices by atomic absorption. Varian Instruments at Work. AA-19:1, Varian Instruments, Palo Alto, CA.

KIRKBRIGHT, G. F., and MARSHALL, M. 1973. Direct determination of phosphorus by atomic absorption flame spectrometry. Anal. Chem. 45:1610.

L'VOV, B. V. 1970. Atomic Absorption Spectrochemical Analysis. Adam Hilger, Bristol, England.

MANNING, D. C., and EDIGER, R. D. 1976. Pyrolysis graphite surface treatment for HGA-2100 sample tubes. At. Absorp. Newsl. 15:42.

MANNING, D. C., and SLAVIN, S. 1969. The direct determination of phosphorus by atomic absorption spectroscopy. At. Absorp. Newsl. 8:132.

ROUTH, M. W. 1979. Direct determination of phosphorus by atomic absorption. Varian Instruments at Work. AA-1:1, Varian Instruments, Palo Alto, CA.

ROWE, C. J. 1973. Food analysis by atomic absorption. Varian Techtron Pty., Ltd., Springvale, Australia.

SIMPSON, G. R., and BLAY, R. A. 1966. Rapid method for the determination of the metals copper, zinc, tin, iron, and calcium in foodstuffs by atomic absorption. Food Trade Rev. 36:35.

[Received January 21, 1983. Accepted May 9, 1983]