Table II. Effect of Storage Time on Residues of Maleic Hydrazide in Field-Treated Potatoes

duration of storage, weeks	maleic hydrazide found,[a] ppm
2	4.6 ± 0.4
3	3.3 ± 0.7
4	4.7 ± 0.9
5	3.0 ± 0.4
6	4.0 ± 0.7
7	3.9 ± 0.7
8	3.3 ± 0.9

[a] Values are the means ± SE of determinations performed on four samples of potatoes harvested 7 weeks after treatment.

Table III. Recovery of Hydrazine from Potato Fortified with Hydrazine Sulfate

hydrazine added, ppb	hydrazine found,[a] ppb	recovery, %
0	0.2	
5	4.4	88
10	9.0	90
25	24.8	99
100	100	100

[a] Values are uncorrected for the blank.

hydrazide decreased that of the glucoside increased until after 4 weeks both were at similar concentrations (Frear and Swanson, 1978). Thus, if potato metabolized maleic hydrazide in a manner similar to tobacco, measurable residues would be expected to be found.

The recoveries of hydrazine added as hydrazine sulfate to samples of untreated potato are given in Table III. Satisfactory recoveries were obtained from 5 to 100 ppb of added hydrazine. No detectable hydrazine was found in tubers sampled at 3 and 9 weeks after treatment using this method. Similarly, no hydrazine was detectable in potatoes stored for 2- or 6-week intervals.

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William H. Newsome

Food Research Division
Health Protection Branch
Food Directorate
Department of National Health and Welfare
Ottawa, Ontario, Canada K1A 0L2

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A Simple and Rapid Colorimetric Method for Phytate Determination

A rapid colorimetric procedure is described for determination of phytate based on the reaction between ferric ion and sulfosalicylic acid. Determination of the phytate content of a variety of cereals, legumes, and oilseeds demonstrates the simplicity of this method compared to the cumbersome digestion and colorimetric method for measuring liberated phosphorus.

The majority of phosphorus in plant seeds is present as phytate, inositol hexaphosphate (Asada et al., 1969; Erdman, 1979; Oberleas, 1971). The reduced availability of zinc associated with plant protein diets is attributed primarily to binding by phytate (Atwal et al., 1980; O'Dell and Savage, 1960; Shah et al., 1976). Determination of phytate has been based on its precipitation as insoluble ferric phytate in acid solution (Ellis et al., 1977; Heubner and Stadler, 1914; Makower, 1970; Oberleas, 1971; Wheeler and Ferrel, 1971). Harland and Oberleas (1977) eliminated the precipitation step and extracted phytate directly with 1.2% HCl which was then eluted through an ion-exchange resin to separate inorganic phosphorus. The eluted phytate was subsequently digested with concentrated H_2SO_4 and concentrated HNO_3 and the liberated phosphorus measured colorimetrically. The digestion stage required careful monitoring to minimize losses due to incomplete or excessive digestion. This paper reports a simple and more direct procedure for measuring phytate based on the reaction between ferric chloride and sulfosalicylic acid. These reagents were used by Wade and Morgan (1955) to detect phosphate esters separated by paper chromatography.

EXPERIMENTAL SECTION

Materials. Calcium and sodium phytate were purchased from Sigma Chemical Co. (St. Louis, MO). Amberlite IR-120S resin and AG1-X8 resin were obtained from Rohm and Haas (PA) and Bio-Rad Laboratories (Richmond, CA), respectively.

Preparation of Phytate Standard. Calcium phytate was converted to free phytic acid by the method of Angyal and Russel (1969) using Amberlite IR-120S. The free phytic acid was then digested according to the procedure described by Harland and Oberleas (1977) and liberated organic phosphorus measured colorimetrically (Chen et al., 1956). The amount of phytic acid was calculated from the organic phosphorus by assuming that one molecule of phytic acid contained six molecules of phosphorus. Alternately, sodium phytate can be used as a standard since

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Scheme I. Extraction of Phytate from Plant Material and Measurement by Digestion and Wade Procedures

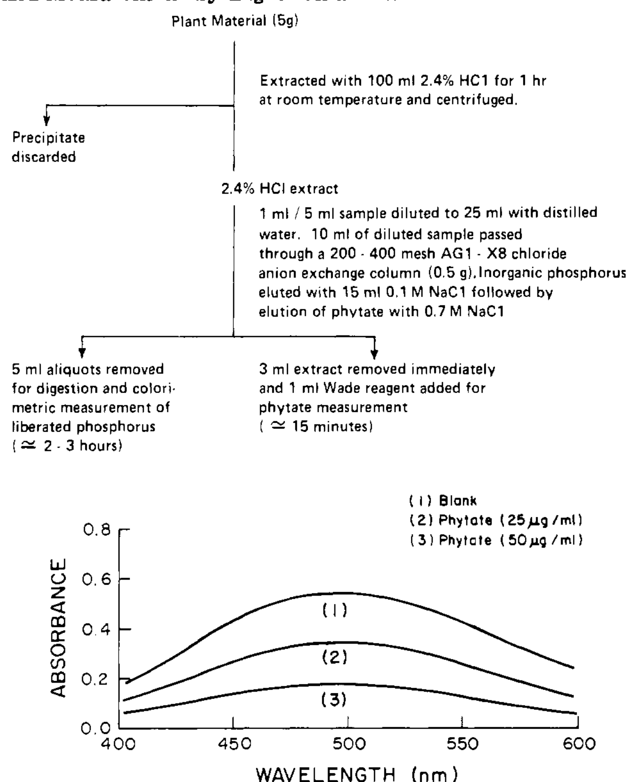

Figure 1. Absorbance spectra of Wade reagent in the presence and absence of phytate.

it is soluble in water and does not require conversion to the free phytic acid form. A series of standard solutions were prepared containing 5–40 µg/mL phytic acid in distilled water. Three-milliliter amounts of the standards were pipetted into 15-mL conical centrifuge tubes with 3 mL of water used as a zero level. To each tube was added 1 mL of the modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water), and the solution was mixed on a vortex mixer for 5 s. The mixture was centrifuged for 10 min and the supernatant read at 500 nm by using water to zero the spectrophotometer. The absorbance spectrum was examined by an SP-800 Unicam spectrophotometer over 450–550 nm while individual absorbances were measured on a Pye Unicam SP 6-300.

Extraction of Phytate from Plant Seeds. Extraction of phytate from various plant sources was carried out by following a modified procedure of Harland and Oberleas (1977) as shown in Scheme I. The initial extraction was carried out with 2.4% HCl (0.65 N) as this proved to be more efficient in extracting total phytate. Elution of the extract through AG1-X8 anion-exchange resin permitted the removal of inorganic phosphorus as well as other interfering compounds. The phytate content was measured by using the Wade reagent and compared to the result of the digestion procedure.

RESULTS AND DISCUSSION

The pink color of the Wade reagent is due to the reaction between ferric ion and sulfosalicylic acid with an absorbance maximum at 500 nm. In the presence of phytate the iron becomes bound to the phosphate ester and unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity (Figure 1). The absorbances of the standards were subtracted from the absorbance of the reagent blank to give the decrease in absorbance values. A linear Beer's law plot was obtained by plotting the decrease in absorbance at 500 nm against phytate concentration in which a coefficient of determination value (r^2) of 1.00 was obtained. Standard curves prepared with purified calcium phytate were similar to those obtained with sodium phytate. Analysis of sodium phytate gave a phytate concentration of 59.0% as compared to a value of 59.9% calculated from data on the bottle. These values were considered similar enough to use the commercial sodium phytate as a standard without further purification.

Table I. Effect of Acid Concentration on Phytate Extraction from Rapeseed[a]

	phytate, %[b]	
HCl, %	Wade method	digestion method
0.6	3.08 ± 0.16	3.08 ± 0.02
1.2	4.01 ± 0.12	4.19 ± 0.06
2.4	4.44 ± 0.12	4.64 ± 0.05
4.8	4.55 ± 0.07	4.66 ± 0.03

[a] Dehulled and defatted. [b] Mean and standard deviation of at least three samples.

Table II. Effect of Dilution of Sample[a] **prior to Ion Exchange on Phytate**[b] **Determinations**

	phytate, %	
dilution	Wade method	digestion method
1:25	0.25 ± 0.06	0.29 ± 0.02
5:25	0.29 ± 0.01	0.29 ± 0.01
10:25	0.22 ± 0.01	0.22 ± 0.01

[a] Sample is bread containing rapeseed flour. [b] Mean and standard deviation of four samples.

The effectiveness of acid concentration in extracting phytate from rapeseed protein concentrate is shown in Table I. It is evident that a more complete extraction is obtained by using 2.4% HCl compared to the 2-h extraction period with 1.2% HCl recommended by Harland and Oberleas (1977). A *t* test at the 5% level showed a significant difference between the 1.2 and 2.4% acid extraction whereas no significant difference was evident between 2.4 and 4.8% HCl extraction. The extraction time was tested by using 2.4% HCl over a 1-h period and was found to give the same results as the 2-h extraction, thus permitting a 50% reduction in extraction time. Recovery of sodium phytate following this procedure was 102 ± 3%, indicating that no losses occurred during the extraction and elution procedures described. The Wade reagent must be added to the 0.7 M NaCl eluant fraction within 0.5 h of passing through the column. Once the reagent is added, however, the mixture remains quite stable.

As may be expected with a procedure that measures an excess of added reagent, some difficulties were encountered with samples containing low levels of phytate. A 1:25 dilution of the extract obtained from a bread containing rapeseed flour was run through the column and gave lower results using the Wade procedure compared to the digestion method (Table II). Adjustment of the sample extract to 5:25 dilution, however, effectively increased the amount of phytate placed on the column and resulted in similar results for both Wade and digestion procedures. Further increasing the amount of extract to 10:25 dilution lowered the results for both methods which may be attributed to high acid concentration altering conditions on the column. It is evident from these results that for samples containing below 1% phytate a dilution of 5:25 is recommended while a 1:25 dilution is adequate for samples

Table III. Phytate Content of a Selected Group of Plant Sources

sample	phytate, %[a]	
	Wade method	digestion method
sunflower protein concentrate	3.66 ± 0.07	3.78 ± 0.02
soybean	1.82 ± 0.09	1.72 ± 0.05
fababean protein concentrate	1.40 ± 0.06	1.48 ± 0.07
pea protein concentrate	1.24 ± 0.05	1.33 ± 0.06
lentil[b]	0.23 ± 0.02	0.27 ± 0.01
wheat flour[b]	0.22 ± 0.01	0.25 ± 0.01

[a] Mean and standard deviation of at least three samples. [b] Using a 5:25 dilution.

containing 1% or more phytate.

Possible interference by phenolic compounds and ascorbic acid reducing $FeCl_3$ was investigated. *p*-Hydroxybenzoic, *trans*-cinnamic, and ferulic acids, which correspond to 50% of the total phenolics in rapeseed, did not react with the Wade reagent at levels 100× that normally found. Ascorbic acid and chlorogenic acid, however, both reacted with the Wade reagent but were not eluted with the phytate fraction and thus did not interfere with the Wade procedure. Phytate levels obtained for a variety of plant materials are listed in Table III. In most cases the Wade values were slightly lower but a *t* test for paired measurements at the 5% level showed no significant differences between the two methods.

This paper demonstrates that the Wade reagent has several distinct advantages over the digestion procedure with respect to the simplicity and rapidity of the procedure. This method is ideal for screening phytate levels in soybean and rapeseed samples for breeding studies. The digestion method is time consuming and quite impractical for monitoring phytate levels in a large number of samples. This study establishes the Wade method as an effective and rapid procedure for phytate determination.

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M. Latta
M. Eskin*

Department of Foods and Nutrition
University of Manitoba
Winnipeg, Manitoba, Canada R3T 2N2

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Identification of 2-Amino-2,6-dideoxygalactose Hydrochloride in a Soil Hydrolysate

A dideoxyamino sugar, 2-amino-2,6-dideoxygalactose hydrochloride (fucosamine hydrochloride), was found for the first time in a soil hydrolysate. It was identified as its alditol acetate by gas–liquid chromatography and mass spectrometry in an eluate fraction following cation-exchange chromatography.

The presence of amino sugars in soils was first demonstrated by Bremner (1949, 1950), who showed their presence in soil hydrolysates by a colorimetric method and paper chromatography. Subsequently, a number of workers have identified glucosamine and galactosamine (Lowe, 1978). The presence of their *N*-acetyl derivatives has also been reported as well as that of another hexosamine, talosamine (2-amino-2-deoxytalose) (Wang and Cheng, 1964). More recently, muramic acid, a component of the peptidoglycan polymer of bacterial cell walls, was found in inorganic soils (Millar and Casida, 1970) and in bog soils (Casagrande and Park, 1978). In view of the relatively large number of amino sugars found in nature (Horton, 1969; Sharon, 1965) and their instability in concentrated hot acid, it has been suggested (Greenland and Oades, 1975) that others exist in the soil. If indeed this is the case, these amino sugars might be components of the "unknown" soil N which represents between 41 and 46% of the total soil N (Sowden et al., 1977). The optimal hydrolytic conditions reported for the analysis of glucosamine and galactosamine, namely, 6 M hydrochloric acid at 100 °C for periods varying between 6 and 12 h, cause significant decomposition of hexosamines, necessitating the use of correction factors ranging from 1.1 to 1.4 (Bremner, 1965). Therefore, less drastic conditions, such as those commonly used in structural determinations of bacterial amino polysaccharides (Perry et al., 1975), were chosen for the analysis of amino sugars in an orthic humic gleysol freed of plant and faunal debris. This communication presents the first report of the identification of 2-amino-2,6-dideoxygalactose hydrochloride (fucosamine hydrochloride) in a soil hydrolysate.

EXPERIMENTAL SECTION

Chemicals. 2-Amino-2,6-dideoxy-L-mannose (rhamnosamine) and 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2,6-dideoxy-D-glucitol were gifts from Dr. M. B. Perry of the National Research Council, Ottawa, Canada.

General Methods. Concentrations were performed under reduced pressure at a bath temperature not exceeding 40 °C. Samples were analyzed by gas–liquid chromatography (GLC) on a Perkin-Elmer 3920 gas chromatograph, equipped with flame ionization and PN detectors. Glass columns (183 × 0.2 cm i.d.) containing

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