Gorsline, G. W., Thomas, W. I., Baker, D. E., *Crop Sci.* **4**, 207 (1964).
Kleese, R. A., Rasmusson, D. C., Smith, L. H., *Crop Sci.* **8**, 591 (1968).
Kramer, C. Y. "A First Course in Methods of Multivariate Analysis," Clyde Y. Kramer, Blacksburg, Va., 1972.
Morgan, D. E., *J. Sci. Food Agr.* **19**, 393 (1968).
National Academy of Sciences, "Nutrient Requirements of Poultry, Sixth Revised Edition," Washington, D. C., 1971.
Rasmusson, D. C., Hester, A. J., Fick, G. N., Byrne, I., *Crop Sci.* **11**, 623 (1971).
Shands, H. L., University of Wisconsin—Madison, private communication, 1974.
Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York, N. Y. 1960, pp 110–111, 313, 317.
Youngs, V. L., *Cereal Chem.* **49**, 407 (1972).

Received for review March 6, 1974. Accepted September 23, 1974. This work was a cooperative investigation between the Agricultural Research Service, USDA, and the Wisconsin Agricultural Experiment Station, Madison. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable. Research supported in part by USDA Cooperative Agreement 12-14-100-10,888, and by the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

Phytic Acid and Other Phosphorus Compounds of Beans (*Phaseolus vulgaris* L.)

George M. Lolas and Pericles Markakis*

Fifty cultivated varieties and lines of mature dry beans (*Phaseolus vulgaris* L.) were analyzed for phytic acid, total P, inorganic P, and other than phytate organic P. The respective concentrations on a dry weight basis were: 0.54–1.58, 0.259–0.556, 0.021–0.044, and 0.050–0.135%. A correlation coefficient of 0.9847 was found between total P content and phytic acid content. A protein–phytate complex was also isolated. The observation was made that 99.6% of the total phytic acid was in a water-soluble form.

Beans, as other seeds, are rich sources of phosphorus (P). According to Earle and Milner (1938), P compounds found in seeds may be classified into four groups: phytates, phosphatides, nucleic compounds, and inorganic P compounds.

Phytins, the mixed Ca and Mg salts of *myo*-inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate), also known as phytic acid, are widespread in nature. They are the principal form of P in many seeds; 60–90% of all the P in these seeds is present as phytic acid (Barré, 1956).

Several physiological roles have been suggested for phytic acid in plants. It may be used as P store (Hall and Hodges, 1966), as energy store (Biswas and Biswas, 1965), or as an initiator of dormancy (Sobolev and Radionova, 1966). Recently, Williams (1970) presented evidence that phytic acid serves only as a source of P and cations for the germinating seed. Asada *et al.* (1968) found that phytate contains over 80% of the total P of mature rice grain and the turnover of phytate P is practically nil in the resting grain. From that they concluded that phytate can be considered a final product of P metabolism in the ripening process.

The animal nutritional importance of phytic acid lies in its ability to chelate several mineral elements, especially Ca, Mg, Fe, Zn, and Mo, and thereby reduce their availability in the intestinal tract (Rackis, 1974; Oberleas, 1973). Bruce and Callow (1934) found that phytic acid reduces the absorption of Ca and is responsible for the rachitogenic properties of certain cereals. Roberts and Yudkin (1960) caused Mg deficiency in rats fed purified diets containing sodium phytate. Evidence was furnished that phytate decreased zinc availability in chicks (O'Dell and Savage, 1960). Earlier, it had been demonstrated that phytic acid interferes with iron absorption in boys (Sharpe *et al.*, 1950).

Phytic acid reacts with proteins to form complex products of varying composition and it has been shown to have an inhibitory effect on the peptic digestion of ovalbumin and elastin (Barré, 1956). This effect is believed to be related to its property to form insoluble combinations with proteins in an acid medium and in a range of pH which corresponds precisely to the optimum for the action of pepsin. It has also been found that elimination of phytic acid from soybean meal extracts is an essential preliminary step to the study of the individual soybean proteins (Smith and Rackis, 1957).

In this work, 50 varieties and lines of mature dry beans (*Phaseolus vulgaris* L.) were analyzed for phytic acid, total P, inorganic, P, and organic P, other than phytic acid. Special attention was directed to a possible relation between total P and phytic acid, and a relation between phytic acid and nitrogen content. A protein–phytate complex in the Sanilac beans (Navy beans) was also isolated and analyzed.

MATERIALS AND METHODS

All the beans analyzed were grown in Michigan and obtained from the Crop and Soil Science Department of Michigan State University.

Total P was determined colorimetrically after digestion of the sample with perchloric acid according to Allen's (1940) method. The determination of inorganic P was based on the colorimetric method of Pons and Guthrie (1946). For the determination of phytic acid a combination of two methods was used. The extraction and precipitation of phytic acid were performed according to the method of Wheeler and Ferrel (1971), while the iron of the precipitate was measured by Makower's (1970) method. A 4:6 Fe/P atomic ratio was used to calculate phytic acid content. Residual P representing nonphytic acid organic P was calculated by subtracting inorganic P and phytic acid P from total P. The protein–phytate complex was isolated from bean flour according to the method of Rackis *et al.* (1961), dried by lyophilization, and analyzed for nitrogen, total P, and inorganic P. The AOAC (1970) Kjeldahl method was followed for the determination of the nitrogen content of beans.

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824.

Table I. Content of Beans in Total P, Phytic Acid, Inorganic P, Organic P Other than Phytic P, and Nitrogen [a]

Varieties	Total P, % dry wt	PA, % dry wt	PA P as % of total P	Inorg P, % dry wt	Inorg P as % of total P	Org P other than PA P, % dry wt	% org P other than PA P, as % of total P	N, % dry wt
1972								
Black turtle soup	0.335	0.70	58.8	0.031	9.3	0.107	31.9	3.36
G.N., Nebraska #1	0.327	0.67	57.7	0.035	10.7	0.103	31.6	3.55
G.N., Tara	0.363	0.83	64.4	0.033	9.1	0.096	26.5	3.43
G.N., U.I. #59	0.333	0.68	57.5	0.034	10.2	0.108	32.3	3.53
G.N., U.I. #61	0.286	0.56	55.1	0.035	12.2	0.093	32.6	3.05
G.N., U.S. #1140	0.283	0.60	59.7	0.031	11.0	0.083	29.4	3.16
N.B., Gratiot	0.369	0.75	57.2	0.033	8.9	0.125	33.8	3.51
N.B., Michelite-62	0.365	0.78	60.2	0.034	9.3	0.111	30.5	3.68
Pinto, #111	0.310	0.64	58.1	0.037	11.9	0.093	29.9	3.71
Pinto, U.I. #114	0.305	0.61	56.3	0.036	11.8	0.097	31.9	3.57
Red Kote	0.373	0.71	53.6	0.038	10.2	0.135	36.2	4.14
R.M., Big Bend	0.308	0.61	55.8	0.035	11.4	0.101	32.9	3.93
R.M., U.I. #34	0.313	0.64	57.6	0.031	9.9	0.102	32.5	3.91
R.M., U.I. #36	0.270	0.54	56.3	0.039	14.4	0.079	29.3	3.43
S.W., U.I. #1	0.259	0.55	59.8	0.031	12.0	0.073	28.2	3.29
S.W., #59	0.340	0.75	62.1	0.021	6.2	0.108	31.7	3.66
1972								
N.B., Sanilac [b]	0.497	1.32	74.8	0.040	8.1	0.085	17.2	4.12
N.B., Seafarer	0.362	0.74	57.6	0.039	10.8	0.115	31.7	3.63
N.B., Seaway	0.448	1.21	76.1	0.034	7.6	0.073	16.4	3.69
1973								
Calif. R.K., #1104	0.497	1.20	68.0	0.041	8.3	0.118	23.8	3.93
G.N., U.I. #31	0.502	1.37	76.8	0.032	6.4	0.084	16.8	3.42
Jules	0.525	1.44	77.2	0.038	7.2	0.082	15.5	3.62
N.B., #20444	0.531	1.47	78.0	0.032	6.0	0.085	16.0	3.70
N.B., #20449	0.528	1.47	78.4	0.034	6.4	0.080	15.2	3.72
N.B., #20454	0.553	1.53	77.9	0.038	6.9	0.084	15.2	3.61
N.B., #20455	0.520	1.33	72.0	0.039	7.5	0.106	20.5	3.48
N.B., #20457	0.534	1.43	75.4	0.038	7.1	0.093	17.5	3.66
N.B., #20459	0.542	1.50	77.9	0.039	7.2	0.081	14.9	3.76
N.B., #20460	0.549	1.58	81.0	0.041	7.5	0.063	11.5	3.91
N.B., #20462	0.507	1.36	75.5	0.035	6.9	0.088	17.6	3.62
N.B., #20463	0.539	1.38	72.1	0.036	6.7	0.114	21.2	3.57
N.B., #20464	0.555	1.52	77.1	0.039	7.0	0.088	15.9	3.75
N.B., #20465	0.498	1.30	73.5	0.027	5.4	0.105	21.1	3.79
N.B., #20466	0.546	1.48	76.3	0.034	6.2	0.095	17.5	3.81
N.B., #20467	0.523	1.41	75.9	0.043	8.2	0.083	15.9	3.77
N.B., #20468	0.538	1.41	73.8	0.041	7.6	0.100	18.6	3.86
N.B., #20469	0.539	1.36	71.1	0.041	7.6	0.115	21.3	3.75
N.B., #20470	0.544	1.35	69.9	0.043	7.9	0.121	22.2	3.79
N.B., #20471	0.556	1.52	77.0	0.038	6.8	0.091	16.2	3.74
N.B., #20472	0.528	1.33	70.9	0.041	7.8	0.112	21.3	3.74
N.B., #20473	0.490	1.29	74.1	0.037	7.6	0.090	18.3	3.79
N.B., #20474	0.553	1.45	73.8	0.040	7.2	0.106	18.9	3.96
N.B., #20475	0.533	1.46	77.1	0.042	7.9	0.080	15.0	3.89
N.B., #20476	0.523	1.33	71.6	0.036	6.9	0.113	21.5	3.82
N.B., #20477	0.532	1.42	75.2	0.039	7.3	0.093	17.5	3.60
N.B., #20478	0.504	1.29	72.1	0.039	7.7	0.102	20.2	3.67
Oregon #58	0.476	1.36	80.5	0.038	8.0	0.055	11.6	3.83
R.M., Big Bend	0.429	1.10	72.2	0.035	8.2	0.084	19.6	3.15
Royal R.K.	0.509	1.30	71.9	0.044	8.6	0.099	19.5	3.65
Swedish Brown	0.483	1.40	81.6	0.039	8.1	0.050	10.3	3.27

[a] Abbreviations used are: P, phosphorus; PA, phytic acid; G.N., Great Northern; N.B., Navy Bean; R.M., Red Mexican; S.W., Small White; R.K., Red Kidney. [b] Twelve analyses in this variety for phytic acid gave a mean and standard deviation of 1.32 ± 0.04.

RESULTS AND DISCUSSION

The results of the analyses (duplicate or triplicate samples), expressed on a dry weight basis, are shown in Table I. The ranges are: for phytic acid, 0.54–1.58%; for total P, 0.259–0.556%; for inorganic P, 0.021–0.044%; and for other than phytic acid organic P, 0.050–0.135%. The phytic acid P represents 53.6–81.6% of the total P, with an average of 69.3%.

The total P content and the phytic acid content of beans were found to be related by the linear regression equation

$$Y = 0.141 + 0.273X$$

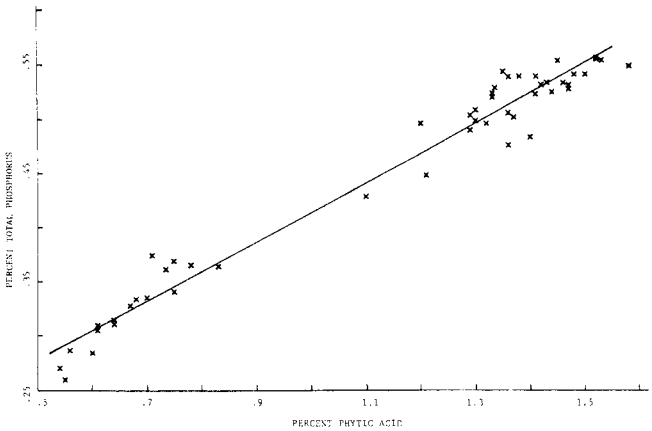

Figure 1. Relationship between total P and phytic acid contents (dry basis) of beans.

where Y is the percentage content in total P and X the percentage content in phytic acid. The correlation coefficient was 0.9847 and the standard error of estimate 0.017%. A graphic presentation of that relationship is shown in Figure 1.

On the basis of these data, it appears that one can estimate the phytic acid content of mature dry beans by determining total P. That is an advantage, especially when a great number of samples are to be analyzed. Phytic acid analysis is time consuming and tedious, whereas total P determination by the colorimetric method is much faster and easier.

The initial bean flour sample yielded 0.47% (dry weight basis) of protein–phytate complex, analysis of which showed that it is composed of 14.2% water, 2.7% nitrogen (or 0.3% of total nitrogen of flour), 12.9% total P (or 12.1% of total P of flour), and 0.024% inorganic phosphorus.

Because of the formation of a protein–phytate complex, it was thought that a relation might exist between protein and phytic acid. A correlation coefficient of 0.3655 was obtained between protein and phytic acid contents indicating no close relation between these constituents of beans.

The following experiment indicates that the phytic acid of beans is present in a soluble form rather than as insoluble phytin. Bean flour was extracted with water first at the 10:1 water/flour ratio for 1 hr under shaking, the slurry was centrifuged, and the sediment was extracted similarly a second time with a water/flour ratio of 5:1. After a second centrifugation, a residue was obtained which upon extraction with 3% trichloroacetic acid (the phytin solvent used in the Wheeler and Ferrel (1961) method) was found to contain approximately 0.4% of the total phytic acid. This agrees with Sandegren's (1948) contention that phytic acid exists principally as the water-soluble sodium–potassium salt in barley.

ACKNOWLEDGMENT

The authors are indebted to Wayne M. Adams for providing the bean samples and encouraging the endeavor. Thanks are also due to the Michigan Bean Commission for supporting in part this research.

LITERATURE CITED

Allen, R. J. L., *Biochem. J.* **34**, 858 (1940).
Asada, K., Tanaka, K., Kasai, Z., *Plant Cell Physiol.* **9**, 195 (1968).
Association of Official Analytical Chemists, "Official Methods of Analysis," Washington, D.C., 1970.
Barré, M. R., *Ann. Pharm. Fr.* **14**, 182 (1956).
Biswas, S., Biswas, B. B., *Biochim. Biophys. Acta* **108**, 710 (1965).
Bruce, H. M., Callow, R. K., *Biochem. J.* **28**, 517 (1934).
Earle, F. R., Milner, R. T., *Oil Soap* **15**, 41 (1938).
Hall, J. R., Hodges, T. K., *Plant Physiol.* **41**, 1459 (1966).
Makower, R. U., *Cereal Chem.* **47**, 288 (1970).
Oberleas, D., "Phytates, in Toxicants Occurring Naturally in Foods," National Academy of Science, Washington, D.C., 1973.
O'Dell, B. L., Savage, J. E., *Proc. Soc. Exp. Biol. Med.* **103**, 304 (1960).
Pons, W. A., Guthrie, J. D., *Ind. Eng. Chem. Anal. Ed.* **18**, 184 (1946).
Rackis, J. J., *J. Amer. Oil Chem. Soc.* **51**, 161A (1974).
Rackis, J. J., Anderson, R. L., Sasame, H. A., Smith, A. K., Van-Etten, C. H., *J. Agr. Food Chem.* **9**, 409 (1961).
Roberts, A. H., Yudkin, J., *Nature (London)* **185**, 823 (1960).
Sandegren, E., *J. Inst. Brew.*, 200 (1948).
Sharpe, L. M., Peacock, W. C., Cooke, R., Harris, R. S., *J. Nutr.* **41**, 433 (1950).
Smith, A. K., Rackis, J. J., *J. Amer. Chem. Soc.* **79**, 633 (1957).
Sovolev, A. M., Rodionova, M. A., *Sov. Plant Physiol.* **13**, 958 (1966).
Wheeler, E. L., Ferrel, R. E., *Cereal Chem.* **48**, 312 (1971).
Williams, S. G., *Plant Physiol.* **45**, 376 (1970).

Received for review July 11, 1974. Accepted September 9, 1974. This manuscript was assigned Michigan Agricultural Experiment Station Journal Article No. 6871.