NOTE

Comparison of Ion-Exchange and Iron Precipitation Methods for Analysis of Phytate

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Some nutritionists believe that phytate decreases the bioavailability to animals and humans of minerals such as zinc and iron (Underwood 1971). Because many foods consumed by livestock and humans contain phytate, accurate methods for phytate determination are necessary. A recent review by Cosgrove (1980) of methods for assaying phytate indicates that in comparative studies, much higher values for phytate were obtained by the iron precipitation procedure than by ion-exchange methods. We recently analyzed wheat bran muffins for phytate by the ion-exchange method and the iron precipitation method. The latter gave phytate values that were about 40% higher than those obtained by the ion-exchange method. Thus, we initiated a study to compare the results by the two methods as applied to 14 other products, including breakfast cereals, wheat bran, bran muffins, soy products, composite human diets, and human fecal samples. In addition, a modified ion-exchange procedure is described that gives results that agree very well with those given by the iron precipitation method.

MATERIALS AND METHODS

Materials

Quick Quaker Oats, Kellogg's Rice Krispies, and Golden Harvest wheat bran were purchased at local stores. Bran muffins were prepared by our Human Study Facility. Human fecal samples were obtained from the study by Morris et al (1980). The composite human diets were self-selected. Soy products were obtained from the USDA Protein Nutrition Laboratory in Beltsville, MD.

Phytate Methods

The ferric ion precipitation procedure (method A) was that described by Ellis et al (1977), except that in the precipitation step the samples were heated for 45 min instead of 20 min. The ion-exchange procedure (method B) was that described by Harland and Oberleas (1977), except the samples were extracted with 2.4% HCl, as suggested by Latta and Eskin (1980), instead of with 1.2% HCl. In method C, the phytate samples were extracted and ferric ion precipitated as described in method A. However, the ferric phytate precipitate was not washed with 0.5 *N* HCl. Instead, the phytate was regenerated by treating the precipitate with 3–8 ml of 1 *N* NaOH, depending on the size of the precipitate, for 1 hr, with occasional stirring with a glass rod. The supernatant containing the phytate was then collected by centrifugation, and the $Fe(OH)_3$ residue was washed twice with demineralized water. The washings and supernatant were combined and made to a definite volume with demineralized H_2O. An aliquot of the supernatant solution with phytate equivalent to the sample used in method B was then chromatographed on the ion-exchange column in the same manner as described in method B.

RESULTS AND DISCUSSION

The phytate of 14 products was determined in duplicate employing the three described procedures. The results are shown in Table I. The phytate values of method A and method B showed poor agreement, with the exception of Rice Krispies and composite human diet samples. When known quantities of phytate were added to ground bran muffins, the recoveries were 97–100% for method A and 62–65% for method B. Harland and Oberleas (1977) analyzed textured vegetable protein samples by the ion-exchange method and an iron precipitation method; the latter gave higher results in every case but one. In contrast to the values of method B, good agreement existed between the phytate values of method A

TABLE I
Comparison of Three Methods for the Analysis of Phytate

Products	Method A[a] Phytic Acid (%)	Method B[b] Phytic Acid (%)	Method B[b] Percent of A Value[d]	Method C[c] Phytic Acid (%)	Method C[c] Percent of A Value[d]
Rice Krispies	0.178	0.180	101	0.175	101
	0.177	0.179		0.182	
Composite human diet					
No. 1	0.400	0.385	96	0.380	95
	0.414	0.398		0.395	
No. 2	0.290	0.272	94	0.277	96
	0.295	0.280		0.285	
Quick Quaker Oats	1.00	0.93	92	0.978	98
	0.99	0.91		0.972	
Soy, concentrate	1.66	1.49	91	1.61	100
	1.61	1.48		1.64	
Golden Harvest Wheat Bran	4.27	3.96	91	4.17	97
	4.37	3.87		4.19	
Wheat bran	2.85	2.46	86	2.80	97
	2.87	2.48		2.76	
Soy, textured	1.92	1.68	86	1.92	99
	1.92	1.65		1.87	
Waldron wheat bran	4.60	4.00	84	...	...
	4.72	3.82		...	...
Soy					
Flour	1.58	1.30	84	1.46	94
	1.52	1.30		1.44	
Isolate	2.04	1.72	82	2.04	98
	2.11	1.67		2.04	
Human fecal					
No. 1	4.01	2.51	62	3.94	98
	4.07	2.49		3.89	
No. 2	3.67	2.21	63	3.46	98
	3.58	2.36		3.63	
Wheat bran muffins	1.26	0.77	60	1.21	96
	1.27	0.76		1.22	

[a] Ferric ion precipitation procedure by Ellis et al (1977).
[b] Ion-exchange chromatography procedure by Harland and Oberleas (1977).
[c] The phytate was precipitated with ferric ion as described in method A; the ferric phytate precipitate was treated with NaOH to regenerate the phytate; the phytate was then chromatographed on ion-exchange as in method B.
[d] The mean phytate value divided by the mean phytate value of method A × 100.

and those of method C. The differences between the phytate values of the two methods were less than 5% in every case but one.

Marrese et al (1961) suggested that higher results might be obtained with the iron precipitation method because of the coprecipitation of other phosphoric esters with phytate. Ellis et al (1977) considered that coprecipitation of inorganic phosphate was a source of error in the Oberleas (1971) iron precipitation method when the samples contained a high phosphate concentration. This source of error was eliminated by incubating the iron precipitate in 0.5*N* HCl for 2 hr at room temperature. Method A included this procedure in the present study. The results in this study suggest that the low values obtained by ion-exchange methods such as method B are due to interfering substance(s) in the acid extract of the product. The results with method C indicate that the interfering substance(s) are either eliminated or the effect minimized when the phytate in the samples is precipitated and regenerated before adding the sample to the ion-exchange column. Cosgrove (1980) suggested that these interfering substance(s) might be proteins. No attempts were made, however, to identify these substances in this study.

CONCLUSIONS

The low phytate values by the previously used ion-exchange method appear to be a result of interfering substances(s) in the acid extract of the product. These interfering substance(s) are either eliminated or the effect minimized when the phytate in the sample is precipitated and regenerated before adding the sample to the ion-exchange column.

LITERATURE CITED

COSGROVE, D. J. 1980. Inositol Phosphates. Elsevier Scientific Publishing Co.: Amsterdam.

ELLIS, R., MORRIS, E. R., and PHILPOT, C. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal. Biochem. 77:537-539.

HARLAND, B. F., and OBERLEAS, D. A. 1977. A modified method for phytate analysis using an ion exchange procedure: Application to textured vegetable proteins. Cereal Chem. 54:827-832.

LATTA, M., and ESKIN, M. 1980. A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28:313-315.

MARRESE, R. J., DUELL, R. W., and SPRAGUE, M. A. 1961. A comparison of three current methods for the analysis of phytin phosphorous. Crop Sci. 1:80–81.

MORRIS, E. R., ELLIS, R., and STEELE, P. D. 1980 Inorganic nutrient balance of humans consuming whole wheat bran vs. dephytinized wheat bran. Fed. Proc. 39:787 (Abstr.)

OBERLEAS, D. 1971. The determination of phytate and inositol phosphates. Methods Biochem. Anal. 20:87.

UNDERWOOD, E. J. 1971. Trace Elements in Human and Animal Nutrition, 3rd ed. Academic Press: New York.

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