NOTE

Appropriate Resin Selection for Rapid Phytate Analysis by Ion-Exchange Chromatography

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The study of Harland et al[2] on phytate analysis of foodstuffs using ion-exchange chromatography indicated wide variation in the phytate concentrations determined by six laboratories despite similar analytical techniques and identical food samples. The coefficient of variation among the laboratories ranged from 16 to 44% for different foodstuffs when AG1-X8 (100–200 mesh) anion-exchange resin was used. Several investigators consulted our laboratory about poor recoveries of phytate from this resin, but discussion with others indicated recovery was satisfactory.

This study compared the recoveries of phytate from different lots of ion-exchange resin and determined the effects of particle size and cross-linkage on quantitative recovery of purified phytate.

MATERIALS AND METHODS

Materials

Seven different lots of AG1-X8 (100–200 mesh), three lots of AG1-X8 (200–400 mesh), and three lots of AG1-X4 (100–200 mesh) Bio-Rad anion-exchange resins were obtained from five laboratories. Sodium phytate was purchased from Sigma Chemical Co. (St. Louis, MO). The ion-exchange columns were the same as described by Ellis and Morris (1983,1984[3]).

Reagents

Disodium ethylenediamine tetraacetate (EDTA)-NaOH solution and sulfonic acid reagent were prepared as described by Ellis and Morris (1983). To prepare molybdate solution, 12.5 g of ammonium molybdate and 50 ml of 10*N* H_2SO_4 were added to a 500-ml volumetric flask, dissolved, and made to volume with deionized water. Sodium phytate stock solution was prepared with 1.10 g of sodium phytate added to a 50-ml volumetric flask, dissolved in deionized water, and made to volume. For sodium phytate working solution, 12.5 ml of sodium phytate stock solution was added to a 250-ml volumetric flask and made to volume with deionized water.

TABLE I
Recovery of Phytate from Different Lots (Same Brand) of AG1-X8 Anion-Exchange Resin

	Percent of Phytate Recovery[a]		
Lot	100–200 Mesh	Lot	200–400 Mesh
26770	62.7 ± 0.8	18707	97.7 ± 1.2
24076	66.8 ± 1.2	22826	97.6 ± 0.7
23003	73.3 ± 2.3	25607	97.0 ± 0.5
20169	79.2 ± 1.7		
20193	84.1 ± 2.3		
22F0366	90.9 ±1.8		
16390	92.1 ± 1.2		

[a] Means ± SD; $n \geq 3$ trials.

TABLE II
Effect of Equilibration on Recovery of Phytate from Ion-Exchange Resins AG1-X8 (100–200 Mesh)

	Percent Recovery	
Lot	No Equilibration	Equilibrated 1.5 hr
26770	62.7 ± 0.8	98.1 ± 0.6
24076	66.8 ± 1.2	97.7 ± 1.0
23003	73.3 ± 2.3	98.0 ± 1.3
20169	79.2 ± 1.7	97.4 ± 1.7
20193	84.1 ± 2.3	98.8 ± 1.5
22F0366	90.9 ± 1.8	98.1 ± 1.7
16390	92.1 ± 1.2	98.6 ± 0.5

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[2] Harland, B. F., Albert, R. H., Harwood, J. P., and Oberleas, D. 1981. A collaborative study: Phytate determination by ion-exchange chromatography. Presented in part at the AOAC 95th Annual Meeting, Oct. 19-22, 1981, Washington, DC.

[3] Ellis, R., and Morris, E. R. 1984. Variability of ion-exchange resins in phytate analysis. Presented in part at the AOAC 98th Annual Meeting, Oct. 28, 1984, Washington, DC.

TABLE III
Effect of Cross-Linkage of Anion-Exchange Resin (100-200 Mesh) on Recovery of Phytate

Cross-Linkage	Number of Lots[a]	Percent Recovery[b] (Range)
AG1-X8	7	78.5 ± 11.5 (62.7–92.1)
AG1-X4	3	98.0 ± 1.1 (97.4–98.5)
AG1-X2	1	97.8 ± 0.8

[a]Same brand.
[b]Mean ± SD of three or more samples/lot; no column equilibration.

Procedure for Phytate Analysis

The ion-exchange columns were prepared as described by Harland et al (1977). A 1-ml aliquot of the phytate solution was added to a 25-ml stoppered, graduated cylinder and made to volume with deionized water. The contents of the cylinder were mixed and poured quantitatively onto the ion-exchange column, and the eluate was discarded. The column then was eluted with 15 ml of 0.1*M* NaCl, and the eluate was discarded. The phytate was then eluted from the column with 15 ml 0.7*M* NaCl, and the entire 0.7*M* NaCl fraction was collected in a 100-ml Kjeldahl flask. Concentrated H_2SO_4 (0.5 ml), concentrated HNO_3 (3 ml), and three glass beads were added to each digestion flask. The mixture was digested as described by Ellis and Morris (1983). The phosphorus in the digest was determined by the method of Fiske and Subbarow (1925). The phytic acid was calculated from the phosphorus on the assumption that one molecule of phytic acid contained six atoms of phosphorus. The percent recovery of added phytic acid was calculated as follows:

$$\% \text{ Recovery} = \frac{\mu\text{g Phytic acid found in digestion from column} \times 100}{\mu\text{g Phytic acid found in digestion of 1 ml of the phytate working solution}}$$

RESULTS AND DISCUSSION

The recovery of phytate from different lots of ion-exchange resin is shown in Table I. There was a wide range of recoveries of phytate for the seven lots of AG1-X8 (100–200 mesh) anion-exchange resins. However, there was little variation in the phytate recoveries from the three lots of AG1-X8 (200–400 mesh). In contrast to the 100–200 mesh resins, phytate recoveries from the 200–400 mesh resins were quantitative. Harland and Oberleas (1977) also showed that purified phytate could be recovered quantitatively from AG1-X8 (200–400 mesh) anion-exchange resin. However, their reason for using the 100–200 mesh in the collaborative study was to decrease the elution time of the column. Flow rates of the 100–200 mesh and 200–400 mesh resin are 1.4 and 0.7 ml/min, respectively, under our conditions. The larger particles increased the rapidity of the column flow, but accuracy was poor.

To determine if additional eluant would elute purified phytate quantitatively from AG1-X8 (100–200 mesh) resin, the columns were eluted with five 15-ml volumes of 0.7*M* NaCl. Three to four 15-ml volumes of 0.7*M* NaCl eluted 97% of the phytate. However, when the columns were stoppered and allowed to stand overnight in 0.7*M* NaCl, the phytate was eluted quantitatively with just one 15-ml volume. Figure 1 and Table II indicate that if the columns were allowed to equilibrate in the eluting solution for 1.5 hr, the recoveries of phytate from the AG1-X8 (100–200 mesh) resin were also quantitative. The equilibration, however, had little effect when

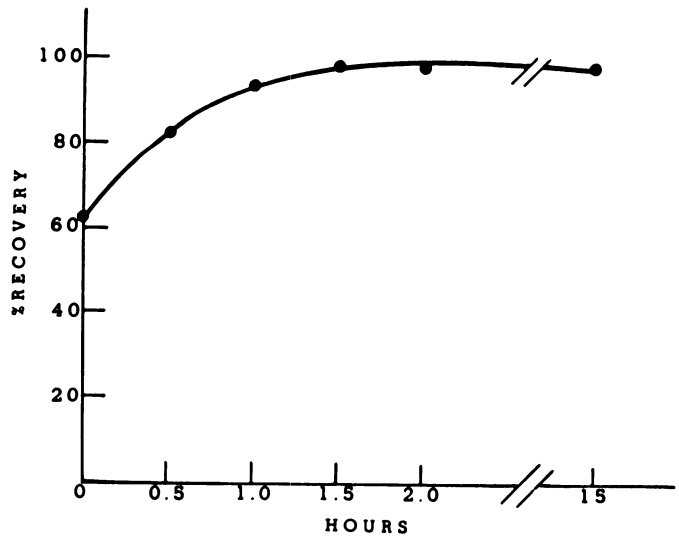

Fig. 1. Effect of equilibration time on phytate recovery from anion-exchange resin AG1-X8 (100–200 mesh, lot 26770).

the pH of the phytate solution added to the ion-exchange column was below 6.0. The recoveries were quantitative in the pH range between 6.0 and 9.0. In the equilibration step, part of the phytate was eluted from the column with 3 ml of 0.7*M* NaCl, the stopcock was closed for 1.5 hr, and the column was then eluted with another 12 ml of 0.7*M* NaCl. Both 0.7*M* NaCl fractions were collected in the same digestion flask and digested for phosphorus analysis. The equilibration step resulted in good phytate recoveries from all seven lots of the AG1-X8 (100–200 mesh) anion-exchange resins, however, it is time-consuming. The data in Table III indicate that if the cross-linkage was decreased from AG1-X8 to AG1-X4, the 100–200 mesh resins gave quantitative phytate recoveries without going through the equilibration step. An advantage of using AG1-X4 (100–200 mesh) over AG1-X8 (200–400 mesh) is rapidity. As reported by Ellis and Morris (1983), the addition of the EDTA-NaOH to the extract of some products such as wheat bran muffins is essential for quantitative recovery of the phytate from the ion-exchange resins, including the AG1-X4 (100–200 mesh). The phytate values for wheat bran muffins were 1.23 and 0.7% with and without EDTA, respectively.

CONCLUSION

This study demonstrated that AG1-X8 (100–200 mesh) anion-exchange resin is not appropriate for phytate analysis, because phytate does not elute quantitatively. There were also large variations in different lots of the AG1-X8 (100–200 mesh) resins. The most appropriate resin for both accuracy and rapidity appeared to be AG1-X4 (100–200 mesh). Our results also indicated that each lot of resin should be carefully checked for quantitative recovery with purified phytate.

LITERATURE CITED

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