PHYTIC ACID (MYOINOSITOL HEXAPHOSPHATE) AND PHYTASE ACTIVITY IN FOUR COTTONSEED PROTEIN PRODUCTS[1]

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ABSTRACT

Phytic acid, as myoinositol hexaphosphate (g/100 g), found in each product as received was 2.86 in a glanded flour, 4.29 in a glandless flour, 3.35 in an air-classified glandless flour, and 2.49 in toasted seed kernels. The glandless flour was significantly higher in phytic acid than the glanded flour (5% level) and than the seed kernels (1% level). On a defatted basis, the glanded flour had a much lower level of phytic acid than the other products. The glanded flour was the only product that did not exhibit phytase activity. The amount of inorganic phosphate released by the glandless air-classified flour during enzyme activity was significantly higher (1% level) than the amounts released by either the glandless flour or the toasted kernels.

The purpose of this study was to determine the amount of inositol hexaphosphoric acid and the relative phytase activity in several cottonseed products that are produced primarily for their protein content. This information is of interest as a guide for evaluating the nutritive value of these products as protein supplements. Concern with the antinutritional properties of phytic acid stems from its ability to combine with divalent calcium, iron, and zinc, often rendering the ions metabolically unavailable to the animal and inducing deficiencies. Zinc deficiencies in chickens (1,2) and man (3), magnesium deficiency in man (4), and calcium deprivation in both animals and man (5) have been induced or aggravated by high dietary levels of phytate phosphorus. Since inositol hexaphosphate reacts with cations and proteins, knowledge of the amount of inositol hexaphosphate in a protein concentrate might also help to anticipate or explain some of the functional properties of the product.

Two developments have spurred renewed interest in cottonseed as a food. Breeding has produced glandless strains of cotton, completely eliminating the gossypol (6,7). The second innovation is the Liquid Cyclone Process, which greatly reduces the amount of gossypol in glanded varieties (7).

Any evaluation of a food suspected to have a high phytic acid content must take into consideration the phytase activity as well as the actual amount of phytic acid present. Thus, though wheat, rye, oats, and corn contain similar amounts of phytate phosphorus, both rye and wheat contain enough phytase to hydrolyze a large portion of their phytate during the leavening of bread (8), but oats and corn have low levels of phytase and lose little phytic acid during breadmaking (9).

MATERIALS AND METHODS

Cottonseed Products

Four food grade high protein cottonseed products were analyzed: 1) a glanded flour (55% protein), 2) a glandless flour (56%), 3) a glandless air-classified flour (61%), and 4) glandless toasted cottonseed kernels (33%). (Protein values were

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supplied by the processors.) The glanded flour, Proflo, was supplied by Traders Oil Mill Co., Fort Worth, Tex. The glandless flour and the glandless air-classified flour were provided by the Food Protein Research and Development Center, Texas A&M University. The cottonseed kernels were distributed by Blaine Richards and Co., Inc., Lake Success, N.Y.

Each of the cottonseed products was thoroughly mixed on arrival, samples were taken, and all products stored at −25° C. The cottonseed kernels to be analyzed were hand ground to 25 mesh before storage.

Methods

The fat extraction method was essentially that of Pons *et al.* (10). Dry weight was determined by the AOAC method (11), under a partial vacuum, with temperatures near 100° C. Acid extractions and phytic acid determinations were carried out using the method of Wheeler and Ferrel (12). Optimum temperature for cottonseed phytase activity is not known. Phytases from different sources have optimum temperatures from 45° to 56° C (13). Phytase activity in each of the products was estimated by the method of Peers (14) at 45° C. The digestion method of Pons *et al.* (10) was used prior to determination of total inorganic phosphorus. Phosphorus was measured before and after incubation by the Pons and Guthrie method (15).

RESULTS AND DISCUSSION

Phytic Acid

The values obtained for phytic acid, as myoinositol hexaphosphate, are given in Table I. The glandless flour had a significantly higher phytic acid content than either the glanded flour (5% level) or the toasted kernels (1% level). Differences between the other products were not significant at the 5% level.

Oil content found was 5.4% for the glanded flour, 4.8% for the glandless flour, 9.9% for the glandless air-classified flour, and 34.8% for the toasted kernels. The level of phytic acid in the defatted toasted kernels indicates that the phytic acid in the kernels, as received, was not associated with the fat fraction. On a defatted basis, only the glanded flour showed a considerably lower amount of phytic acid.

Nelson *et al.* (16) reported the level of phytic acid in a 50% protein cottonseed meal to be 3.30 g/100 g. This is somewhat lower than the value of 4.29 found in this study for the glandless flour (56% protein) but comparable to the 2.86 g/100 g for the glanded flour (55% protein). Varietal differences between the seeds from which products are made would be expected to influence the amount of phytic acid in the end product (17). There may have been varietal differences between the products used in this study. These differences would contribute a source of variation to comparisons of phytic acid content between products.

In the cottonseed, globoid bodies, containing the phytic acid, are enclosed within aleurone grains which have a protein storing function. The phytic acid in the cottonseed would not be expected, on the basis of structure, to be lost during the production of the refined cottonseed products, particularly when protein content is to be maximized. Martinez *et al.* (7) state that in dry air classification of the glandless cottonseed flour, the aleurone grains remain intact, thereby also concentrating the phytic acid-containing globoid bodies. However, in our study, phytic acid values for the glandless flour (56% protein) did not differ significantly from the glandless air-classified flour (61% protein).

To evaluate the importance to the diet of the phytic acid contributed by a cottonseed product, it is necessary to consider both the amount of phytic acid present and the amount of the product recommended for use as a protein supplement or functional improver.

Cosgrove (18) and Foy *et al.* (19) reported an increased need for iron when phytic acid phosphorus was as high as 40% of the dietary phosphorus. The amount of cottonseed flour generally recommended for use is 1–3% of the total formula weight of the mixture (20)[2,3]. When such low levels of the product are used, statistically significant differences in phytic acid content may not be nutritionally significant. Newer formulations using cottonseed protein products are recommending levels of incorporation as high as 30% of the formula weight. The nutritional effect of each of these products should be evaluated at the level to be used.

Of the products tested in this study, only the toasted kernels would be likely to be ingested as purchased. The amount of these nuts needed to reach the hypothetical 40% level is small (approximately 6.5 tablespoons for an 11 to 18 year old, 1973 Recommended Dietary Allowances, National Research Council). Efforts should be made to explore ways to lower the amount of phytic acid in the

[2]Traders Oil Mill Company, Protein Division. Proflo's function in cake doughnuts. n.d. Product data sheet No. 106.

[3]Traders Oil Mill Company, Protein Division. Biscuit and cracker uses of Proflo and Cinacoa. 1968. Product data sheet No. 109.

TABLE I
Phytic Acid, as Myoinositol Hexaphosphate, in Four Cottonseed Products (g/100 g)

Product	As Received	Defatted	Dry Weight Basis
Glanded flour	2.86 ± 0.56	2.44 ± 0.35	2.94
Glandless flour	4.29 ± 0.30	4.25 ± 0.53	4.78
Glandless air-classified flour	3.35 ± 0.35	3.94 ± 0.89	3.86
Toasted kernels	2.49 ± 0.03	4.01 ± 0.48	2.57

TABLE II
Changes in Inorganic Phosphate and Estimates of Phytic Acid Loss in Four Cottonseed Products with Moist Heat Treatment

Products	Inorganic Phosphate μg/100 mg product: Before heating	After heating	Change	Estimate Phytic Acid Lost during Heating %
Glanded flour	100 ± 2	98 ± 4	−2	0
Glandless flour	100 ± 6	178 ± 10	+78	6
Glandless air-classified flour	56 ± 6	184 ± 12	128	13
Toasted kernels	44 ± 6	126 ± 8	82	12

toasted kernels or to encourage their incorporation in food systems that would promote phytase activity, rather than their use as a snack food.

Phytase Activity

In the aqueous system used, of all the products tested, only the glanded flour did not exhibit phytase activity (Table II). The moist heat treatment used for the production of the glanded flour may have resulted in enzyme inactivation. The low level of phytic acid found in the glanded flour (Table I) indicates that some enzyme activity may have occurred prior to enzyme inactivation. Exact times and temperatures used in the process are not available.

The higher enzyme activity in the glandless air-classified flour may be due in part to its higher protein content. The enzyme activities exhibited by the three glandless products are computed in terms of the proportion of phytic acid destroyed (Table II). For this estimation, the assumptions were made that the loss of six inorganic phosphate groups represented the destruction of one molecule of myoinositol hexaphosphate and that all the phytic acid present before moist heat treatment was the hexaphosphate ester of phytic acid. Since it is not necessary to completely dephosphorylate the phytic acid to render it incapable of metal chelation, these assumptions make the figures given for loss of phytic acid conservative.

The phytic acid content of cottonseed products should not be overlooked when they are to be used as protein supplements. For each food system to be used, the amount of phytic acid and phytase activity in the cottonseed product, the level of incorporation, and the type and length of heat to be applied should be considered.

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