hypogea L.) by Flavonoid Analysis". *Peanut Sci.* **1983**, *10*, 40–43.
Drexler, J. S.; Williams, E. J. "A Non-Destructive Method of Peanut Pod Maturity Classification". *Proc. Am. Peanut Res. Educ. Soc.* **1979**, *11*, 57.
Pattee, H. E.; Johns, E. B.; Singleton, J. A.; Sanders, T. H. "Composition Changes of Peanut Fruit Parts During Maturation". *Peanut Sci.* **1974**, *1*, 57–62.
Pendse, R.; Rama Rao, A. V.; Venkataraman, K. "5,7-Dihydroxychromone from *Arachis hypogea* Shells". *Phytochemistry* **1973**, *12*, 2033–2034.
Sanders, T. H.; Schubert, A. M.; Pattee, H. E. "Maturity Methodology and Post Harvest Physiology". In *Peanut Science and Technology*; Pattee, H. E., Young, C. T., Eds.; American Peanut Research and Education Society: Yoakum, TX, 1982.
Schenk, Ray. V. "Development of the Peanut Fruit". Technical Bulletin N.S. 22, Georgia Agricultural Experimental Station, May 1961.
Williams, E. J.; Drexler, J. S. "A Non-Destructive Method for Determining Peanut Pod Maturity". *Peanut Sci.* **1981**, *8*, 134–141.
Williams, E. J.; Monroe, G. E. "Impact Blasters for Peanut Maturity Determination". *Trans. Am. Soc. Agric. Eng.* **1986**, *29*, 263–267.

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Removal of Phytic Acid from Soybean and Cottonseed Meals

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Soybean and cottonseed meals were subjected to various treatments with enzymes, heat, and chemicals, and their effects on hydrolysis and removal of phytic acid were studied. Soybean meal and cottonseed meal contain about 2.2 and 4.4% (w/w) phytate, of which 60 and 50%, respectively, were in water-soluble forms. The water-insoluble portion of the phytate was further hydrolyzed and removed by extraction with acid, application of enzymes, precipitation by divalent cations, and autolysis by endogenous phytase. Washing with 1 N HCl removed about 87% of phytate in soybean meal, while treatment with cationic agents had little effect on removing phytate from the plant seed. Coapplication of phytase with cellulose showed a synergistic effect in the hydrolysis of phytate. Incubating cottonseed meal in the presence of water in the temperature range 30–60 °C significantly reduced phytate content in the seed meals.

Plant seeds contain certain antinutrient substances that have an adverse effect on the nutritive properties of the seed protein. Phytic acid in soybean and cottonseed meal is an example. Phytic acid and its derivatives are known to bind essential dietary minerals, thus making them unavailable or only partially available for absorption by animals. Soybean and cottonseed meals are the major protein supplement in poultry feeds and also are a source of phosphorus. However, two-thirds of soybean meal phosphorus is bound as phytate and is unavailable for poultry (Whitaker and Brunnert, 1977). Although phytase activity has been shown to be present in the small intestine of various animals, its activity is not sufficient to utilize dietary phytic acid. Hydrolysis and/or removal of phytic acid prior to animal consumption would increase the availability of inorganic phosphorus and other minerals in the animal diet. Thus, efforts have been made to remove or reduce phytic acid level in seed protein by application of microbial enzymes (Han, 1988; Whitaker and Brunnert, 1977; Liener, 1977; Nelson et al., 1968; Rojas and Scott, 1969), cationic metal salts (Brooks and Morr, 1984), and autolysis (hydrolysis of phytate by endogenous phytase) (Chang and Schwimmer, 1977). Although beneficial effects of the enzyme treatment were evident, the high cost of enzyme production and lack of a practical method for enzyme application were cited as limiting factors in using the enzyme in animal diets (Han, 1988). The objective of this study was to investigate the effect of various treatments (washing, autolysis, enzyme and cation applications) in reducing phytate content in soybean and cottonseed meals.

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MATERIALS AND METHODS

Materials. Soybean meal and cottonseed meals were obtained from a local feed store and stored at 18 °C and 50% relative humidity until used. The moisture content of the meals was about 11%, and the total phytate contents of the soybean meal and cottonseed meal were 2.27 and 4.40%, respectively. Phytase, cellulase, hemicellulase, and proteinase (bromelain) were obtained from Sigma Chemical Co., St. Louis, MO.

Treatment. Two-gram portions of soybean and cottonseed meals were mixed with 30 mL of a solution containing 1 unit each of various enzymes, a combination of such enzymes, or various concentrations of cationic salts. The substrate mixed with the same amount of water was used as a control. The enzyme solutions were adjusted to pH 5.4 with acetate buffer, whereas the pH of the mineral solutions was not adjusted. The mixtures were then incubated at room temperature (about 25 °C) for a predetermined time period. For study of autolysis, 2 g of substrate was mixed with 2 or 30 mL of water and the resultant mixture incubated at various temperatures for the preset time period. Sodium hypochlorite (0.2%) was added to soybean meal to prevent microbial growth during the incubation period. At the end of incubation, phytate content in water-soluble and water-insoluble portions was determined. The phytate loss (%) was calculated on the basis of the total amount of phytate lost by the treatment compared to that of untreated control. All the treatments were replicated (two to five times), and the values reported are the average of the replicated samples.

Table I. Extraction of Phytic Acid in Soybean Meal by Different Acids[a]

acid (concn, N)	pH	phytate extracted, % of total solid
control, H_2O	5.0	1.48
HCl (0.01)	2.33	1.41
HCl (0.1)	1.35	0.30
HCl (1.0)	0.49	1.92
H_2SO_4 (0.01)	2.20	1.47
H_2SO_4 (0.1)	1.38	0.33
H_2SO_4 (1.0)	0.62	1.52
H_3PO_4 (0.01)	2.50	1.41
H_3PO_4 (0.1)	1.95	0.76
H_3PO_4 (1.0)	1.36	0.43
HCOOH (0.01)	3.30	1.48
HCOOH (0.1)	3.12	0.75
HCOOH (1.0)	2.42	0.24

[a] A 2-g portion of substrate was mixed with 30 mL of acid solution and shaken for 1 h at rom temperature. Solubilized phytic acid was collected by centrifugation and analyzed by the method described in Materials and Methods.

Determination of Phytic Acid. Phytic acid in soybean and cottonseed meals was extracted and determined according to the modified method of Latta and Eskin (1980) as follows: A 2-g portion of substrate was mixed with 30 mL of water, shaken constantly for 1 h at 25 °C, and centrifuged to remove water-soluble phytate (supernatant 1). To the precipitate was added 30 mL of 0.65 N HCl, and the mixture was placed on a shaker for 1 h at 25 °C and centrifuged to remove the acid-soluble phytate (supernatant 2). The two supernatants were then eluted through an anion–exchange resin (Dowex 1X-8, 200–400 mesh, chloride form; Biorad Laboratory, Richmond, CA), first with 0.1 M NaCl (to remove inorganic phosphorus and other interfering compounds) and then with 0.7 M NaCl (to collect phytic acid). The phytate content was measured by using the modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water). The degree of color change in the Wade reagent by the sample was measured by reading optical density at 500 nm and correlated to the concentration of phytic acid. Sodium phytate (Sigma) was used as a standard for estimating phytic acid. Acetate buffer was used throughout the experiment because the citrate and phosphate buffer interfered with the color development in the Wade reagent.

RESULTS AND DISCUSSION

Extraction of Phytic Acid. The effectiveness of different acids in removing phytic acid from soybean meal was investigated. The results (Table I) show that 1 N HCl extracted the highest amount (about 87%) of phytic acid from soybean meal, while 0.01 N concentrations of HCl, H_3PO_4, HCOOH, and H_2SO_4 removed about 67% of phytic acid from soybean meal, which is almost the same level as that obtained by water. There was no clear relationship between acid concentration and phytate extracted. For example, in the case of strong acids (HCl, H_2SO_4), more phytic acid was extracted with 1 and 0.01 N acids than with 0.1 N concentration, whereas for weak acids (H_3PO_4, HCOOH) higher levels of phytic acid were extracted as the concentration of acid decreased. Acidity (acetate buffer, pH 3.7–5.6) and temperature (25–50 °C) of the extracting medium, time (1–24 h), and particle size (diminution by a Waring blender) of the substrate had little effect on the amount of phytate extracted.

Effect of Enzyme Treatment. The ability of phytic acid to bind metal ions is lost when the phosphate groups are hydrolyzed through the action of phytase enzyme (Nelson et al., 1968). Since plant phytate exists in close association with other cellular components, coapplication of phytase with other enzymes was believed to have some effect on the phytate hydrolysis. For example, phytase treatment resulted in a substantial increase in metabolizable energy of cottonseed meal for chicks (Rojas and Scott, 1969). This was due to the hydrolysis of phytate and its release from the protein, thereby making the latter more readily digestible.

Table II. Enzymatic Hydrolysis of Phytic Acid in Soybean Meal

enzyme	phytate, % of total solid: sup 1[a]	sup 2[b]	total[c]	phytate hydrolyzed,[d] %
control (no enzyme)	1.64	0.60	2.24 ± 0.03	0
phytase	1.02	0.39	1.41 ± 0.02	37.0
hemicellulase	1.67	0.68	2.34 ± 0.01	0
cellulase	1.48	0.65	2.31 ± 0.03	4.9
proteinase	1.71	0.59	2.30 ± 0.04	0
phytase + hemicellulase	0.92	0.37	1.29 ± 0.12	42.4
phytase + cellulase	0.90	0.35	1.25 ± 0.05	44.2
phytase + proteinase	1.06	0.38	1.44 ± 0.06	35.7
phytase + hemicellulase + cellulase	0.90	0.35	1.25 ± 0.06	44.2
phytase + cellulase + proteinase	1.01	0.39	1.39. ± 0.07	37.9
phytase + hemicellulase + cellulase + proteinase	0.92	0.41	1.33 ± 0.03	40.6

[a] One unit each of enzyme in 30 mL of acetate buffer (0.01 M, pH 5.4) was added to 1 g of substrate and incubated for 3 h at 37 °C. [b] Sup 1 designates the water-soluble fraction, and sup 2 represents the water-insoluble phytate. [c] Means and standard deviations of three assays. [d] Percent hydrolyzed was calculated based on the amount of phytate that disappeared after the enzyme treatment compared to the control.

Soybean and cottonseed meals were treated with phytase alone or in combination with other enzymes, such as cellulase, hemicellulase, and proteinase, and the effect of these treatments on phytate hydrolysis was studied. With the application of phytase, 37% of phytate in soybean meal was hydrolyzed during 3-h incubation at 37 °C (Table II). The extent of phytate hydrolysis was further increased by coapplication of cellulase with phytase. Treatment with other enzymes, however, had little effect on the ability of phytate to be hydrolyzed in soybean meal. The highest level (44.2%) of phytate hydrolysis was obtained in the sample treated with a combination of phytase and cellulase. Little or no hydrolysis of phytate occurred in the samples treated with cellulase, hemicellulase, or proteinase alone.

Effect of Cationic Agents. The ability of cationic metals to complex with and precipitate phytate ions was investigated as a possible way to produce phytate-reduced soybean meal. In general, hydrogenated and monovalent salts of phytic acid are water soluble, whereas its divalent and trivalent metal salts are quite insoluble. Treatment of soybean and cottonseed meals with cationic agents was thought to change the solubility of phytate, thus making it more accessible to subsequent enzymatic hydrolysis or other treatments to remove phytate from the seed meals.

Treatment of soybean meals with divalent and trivalent cationic agents at concentrations of more than 0.1% produced little or no water-soluble phytate or increased the levels of water-insoluble phytate (Table III). The chelating effect was diminished as the concentration of the metal ions decreased below 0.1%. Trivalent ion appeared to have more chelating effect than the divalent ion of the same metal. Treatment with monovalent cationic agents produced almost the same level of water-soluble phytate

Table III. Effect of Cations in Phytate Removal from Soybean Mean

cation[a] (%)	phytate, % of total solid: water-sol	water-insol	total	% loss[b]
control, H_2O	1.50	0.77	2.27	0
$FeCl_3$ (1.0)	0	0	0	100
$FeCl_3$ (0.1)	0	0.33	0.33	85.0
$FeCl_3$ (0.01)	1.08	0.74	1.82	17.2
$FeCl_2$ (1.0)	0	0	0	100
$FeCl_2$ (0.1)	0.42	0.30	0.72	67.3
$FeCl_2$ (0.01)	1.50	0.71	2.21	0
$BaCl_2$ (1.0)	0.20	1.50	1.70	22.7
$MgCl_2$ (1.0)	0.50	1.20	1.70	22.7
$CaCl_2$ (1.0)	0	1.60	1.60	22.7
$ZnCl_2$ (1.0)	0	1.40	1.40	36.4
$NaCl_2$ (1.0)	1.54	0.45	1.99	12.3
KCl (1.0)	1.28	0.56	1.84	18.9

[a] A 2-g portion of substrate was mixed with 30 mL of mineral salts solution, and the mixtue was shaken 3 h at room temperature. [b] Percent loss was calculated on the basis of the amount of total phytate recovered after the treatment compared to that of control.

as that obtained by water. The levels of phytate in the treated samples were less than that of untreated sample (control). Treatment of soybean meal with 1.0% level of $FeCl_2$ and $FeCl_3$ completely blocked the phytate, and no residual phytate was detected in either the water-soluble or water-insoluble portion of the material. This is believed to be due to the formation of metal–phytate complex, which is not detected by the analytical method we used, rather than the destruction of phytate per se.

Autolysis. Plant seeds contain endogenous phytase that, upon activation, hydrolyze their own phytate (Chang and °chwimmer, 1977). If proper environmental conditions were provided, the phytate content of plant seed would be reduced by the action of endogenous phytase. To investigate the optimum temperature and time for activation of endogenous phytase, cottonseed meal was mixed with water and incubated at various temperatures (30–60 °C) and the level of phytate in the treated materials was determined. Incubation of cottonseed meal at temperatures between 30 and 60 °C decreased the level of phytate by 11.8–17.7% in 4-h incubation and by 45–54.5% in 16-h incubation (Table IV). When water at 1:1 (substrate to water) ratio was added to soybean and cottonseed meals, the reduction in phytate content was same as when water was added at 1:15 (substrate to water) ratio of water. A certain amount of phytate was diffused out of the seed meal upon prolonged (16-h) incubation and was found in the incubating medium. This was evident because the level of water-soluble phytate (supernatant 1) increased and the water-insoluble phytate (supernatant 2) decreased in the samples incubated at 40 and 50 °C. This leaching effect was not apparent in the samples incubated for 4 h.

CONCLUSION

A majority (more than 50%) of phytic acid in soybean and cottonseed meals was easily removed by washing with water or 1 N HCl. Temperature (25–50 °C) and pH (3.7–5.6) of the extracting media, extraction time (1–24 h), and particle size of the seed meals had little effect on the amount of phytate extracted. Application of exogenous phytase or activation of endogenous phytase further hydrolyzed water-insoluble phytate in the seed meals. Coapplication of phytase with cellulase produced a synergistic effect in hydrolyzing phytate in soybean meal. Almost half of the cottonseed phytate was hydrolyzed by the endogenous phytase upon incubating 16 h at 60 °C. Since most of the cationic agents tested did not significantly increase the amount of water-soluble phytate or reduce water-insoluble phytate, treatment of soybean meals with metal solution did not appear practical.

Table IV. Effect of Temperature and Time on Autolysis of Phytate in Cottonseed Meal

treatment:[a] temp, °C	phytate, % of total solid: sup 1	sup 2	total	% loss
control	2.20	2.20	4.40	0
A. 4-h Incubation				
30	1.77	2.11	3.88	11.8
40	1.74	2.05	3.79	13.8
50	1.66	1.96	3.62	17.7
60	1.69	1.96	3.65	17.0
B. 16-h Incubation				
30	1.46	0.96	2.42	45.0
40	1.69	0.62	2.31	47.5
50	1.76	0.54	2.30	47.7
60	1.07	0.93	2.00	54.5

[a] A 2-g portion of substrate was mixed with 30 mL of water and incubated 4 and 16 h at different temperatures. The control was untreated cottonseed meal.

Registry No. $FeCl_3$, 7705-08-0; $FeCl_2$, 7758-94-3; $BaCl_2$, 10361-37-2; $MgCl_2$, 7786-30-3; $CaCl_2$, 10043-52-4; $ZnCl_2$, 7646-85-7; NaCl, 7647-14-5; KCl, 7447-40-7; phytic acid, 83-86-3; phytase, 9001-89-2; cellulase, 9012-54-8; hemicellulase, 9025-56-3; bromelain, 37189-34-7.

LITERATURE CITED

Becker, R.; Olsen, A. C.; Fredrick, D. P.; Kon, S.; Gumbman, M. R.; Wagner, J. R. Conditions for the Autolysis of Alpha-galactosidase and Phytic Acid in California Small Beans. *J. Food Sci.* **1974**, *39*, 766–769.

Brooks, J. R.; Morr, C. V. Phytate Removal from Soybean Proteins. *JAOCS, J. Am. Oil Chem. Soc.* **1984**, *61*, 1056–1059.

Chang, R.; Schwimmer, S. Phytate Removal from Whole Dry Beans by Enzymatic Hydrolysis and Diffusion. *J. Food Sci.* **1977**, *42*, 1098–1101.

Han, Y. W.; Wilfred, A. G. Hydrolysis of Phytate in soybean and Cottonseed Meals by *Aspergillus Ficuum* Phytase. *J. Agric. Food Chem.* **1988**, *36*, 259–262.

Latta, M.; Eskin, M. J. A Simple and Rapid Colorimetric Method for Phytate Determination. *J. Agric. Food Chem.* **1980**, *28*, 1313–1315.

Liener, I. E. Removal of Naturally Occuring Toxicants Through Enzymatic Processing. *Adv. Chem. Ser.* **1977**, *No. 160*, 283–300.

Nelson, T. S.; Shieh, T. R.; Wodzinski, R. J.; Ware, J. H. The Availability of Phytate Phosphorus in Soybean Meal Before and After Treatment with Mold Phytase. *Poultry Sci.* **1988**, *47*, 1842–1848.

Rojas, S. W.; Scott, M. I. Factors Affecting the Nutritive Value of Cottonseed Meal as a Protein Source in Chick Diets. *Poultry Sci.* **1969**, *48*, 819–835.

Whitaker, F.; Brunnert, H. Enzymatic Modification of Protein Applicable to Foods. *Adv. Chem. Ser.* **1977**, *No. 160*, 95–155.

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