Oilseed Phytates: Nutritional Implications

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ABSTRACT

The protein quantity and quality, caloric value, and overall nutrient content of oilseeds are quite good. However, oilseeds are high in phytic acid and contain fiber and perhaps other binding agents which reduce mineral bioavailability from the seeds. Phytic acid, the hexaphosphate of myoinositol, functions as the chief storage form of phosphate and inositol in mature seeds. On a dry basis, whole oilseeds contain about 1.5% while some oilseed protein concentrates can contain over 7.0% of the compound. Phytic acid is a strong chelating agent that can bind mono- and divalent metal ions to form the complex phytate. Published results from numerous animal feeding trials suggest poor bioavailability of minerals such as zinc, calcium, magnesium, phosphorus and possibly iron from diets containing high phytate foods. Recent studies involving the feeding of soy products to rats suggest that zinc is the mineral of most concern as its bioavailability from some soy products is quite low. Prediction of mineral bioavailability from phytate-containing foods is complicated by the complex interactions between the minerals and phytic acid contained in the foods, intestinal and the meal phytase activities, previous food processing conditions (especially pH), digestibility of the foods as well as the physiological status of the consumer of the foods. Very little is known about the chemistry of such interactions. Therefore, most of the emphasis in controlling or reducing mineral binding in oilseed products has been placed upon development of methodology for phytate removal.

INTRODUCTION

True assessment of an individual's or population's mineral status requires more than simple chemical or spectrophotometric analysis of dietary components. Chemical presence of a mineral provides little assurance of its availability for absorption and utilization. Minerals from cereals, oilseeds and other plant foods, in contrast to minerals from animal sources, are in general poorly utilized by man (1). Reduced intestinal uptake of minerals from plant foods exceeds that which might be attributed to the lower digestibility of these foods. Certain plant food components are known to chelate minerals and reduced their absorption. Although numerous studies have been performed to identify these endogenous factors, there is little agreement as to the relative importance of the individual mineral binders. Phytic acid (2-4), various types of dietary fiber (5,6) and the basic amino acids (7) all bind metal ions.

This review is primarily devoted to the chemistry, biological occurrence, nutritional implications and methods of removal of phytic acid from oilseed products. This paper highlights and expands upon some excellent review articles published on the subject (8-13). These papers should be consulted for more extensive literature coverage.

CHEMISTRY OF PHYTIC ACID

The chemical structure of phytic acid, the hexaphosphate of myoinositol, has been continually questioned. At issue has been the isomeric conformation of the phosphate groups within the compound and whether three strongly bound water molecules are incorporated into the structure. Figures 1A and 1B show the most acceptable structures which were suggested by Anderson (14) and Neuberg (15). Potentiometric titration of crystalline sodium phytate shows the presence of six strongly dissociated protons (pk's < 3.5) and six weakly dissociated protons (pk's 4.6-10) (16). These results support the Anderson structure for phytic acid. Brown et al. (17), however, reported that 6 more hydrogens (to total 18) were titratable in aqueous and nonaqueous media. These last six hydrogens were too weakly acidic to be originally titratable in water. This work upholds the Neuberg structure as the correct one. Although additional support for each structure has been published, most evidence points to the Anderson model (8,18-20) as the predominant form in plant materials. However, the possible existence of two phytic acids should not be overlooked (17,20).

As noted by Oberleas (10), the proper chemical designation for phytic acid is myoinositol 1, 2, 3, 4, 5, 6-hexa kis (dihydrogen phosphate) (21). There is some question whether five of the phosphates lie in an axial or in an equatorial plane. Figure 2A shows a structure determined by x-ray crystal analysis (18), while 2B depicts a structure obtained with ^{31}P nuclear magnetic resonance (19). In either case, the structures suggest tremendous chelating potential. For current discussion of the molecular structure

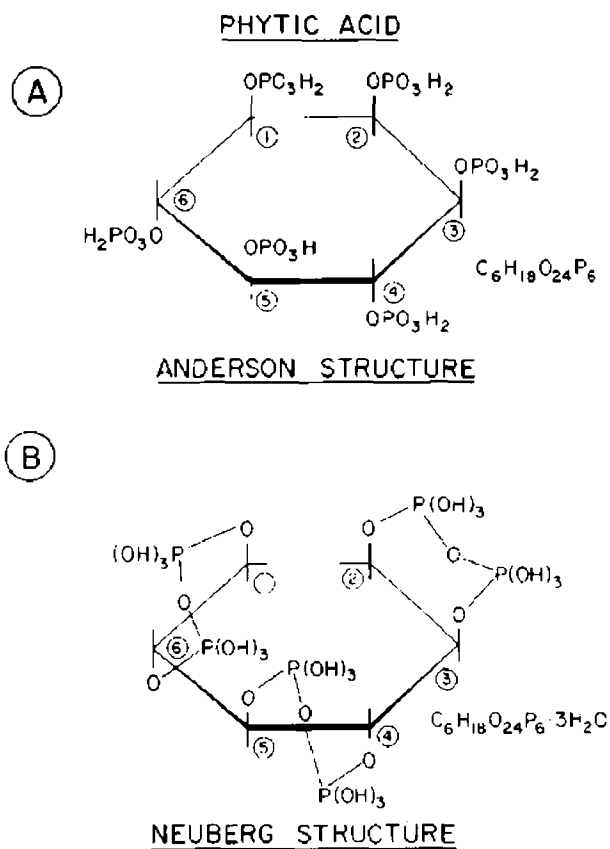

FIG. 1. Proposed structures for phytic acid. Structure 1A suggested by Anderson (14) and structure 1B suggested by Neuberg (15).

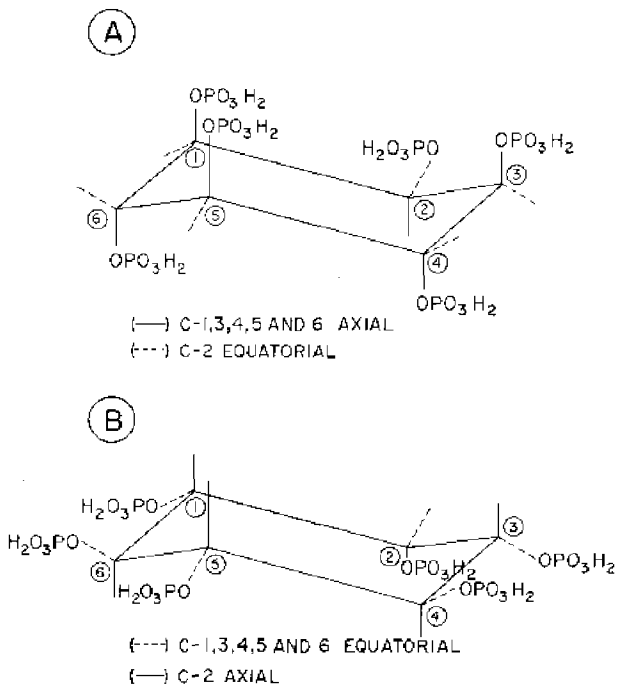

FIG. 2. Proposed conformations of phytic acid. Structure 2A suggested by Blank et al. (18) and structure 2B suggested by Johnson and Tate (19).

of phytic acid see Costello et al. (20).

Weingartner and Erdman (22) recently suggested a partically dissociated Anderson–based structure for phytic acid that might occur at a neutral pH (Fig. 3A). It is apparent that various cations could strongly chelate between two phosphate groups or weakly within a phosphate. Figure 3B is a conjectural depiction of a mixed salt chelate of phytic acid (phytate). The relative binding strengths of various metal ions to phytic acid vary greatly and will be discussed in a later section of this review.

BIOLOGICAL FUNCTION

Phytates are considered the chief storage form of phosphate and inositol in almost all seeds (8,23). The ripening process is characterized by active transport of phosphorus to seeds from leaves and roots. Most of the transported phosphorus is eventually found in phytic acid (23). In cereal grains 60–80% of the total phosphorus is accumulated in phytic acid. Formation of phytic acid during maturation of seeds and tubers is thought to prevent accumulation of excessively high levels of inorganic phosphate (8).

It has been generally assumed that phytic acid is utilized as a source of phosphorus at germination (23). Chen and Pan (24) have reported a 227% increase in phytase activity 5 days after germination of soybeans, while in this same period one variety of pea had a 3700% increase in phytase activity. Phytic acid may also act as a phosphagen (23) during germination. Evidence has been presented to show the occurrence of transphosphorylation to ADP (25) and the presence of an enzyme to catalyze transphosphorylation to GDP in the mung bean (26).

As phytic acid accumulates in various storage sites in seeds and tubers, other minerals apparently chelate to it forming the complex salt phytate. However, no evidence can be presented to support a hypothesis that phytic acid acts as a carrier or storage site for trace minerals during plant ripening (8).

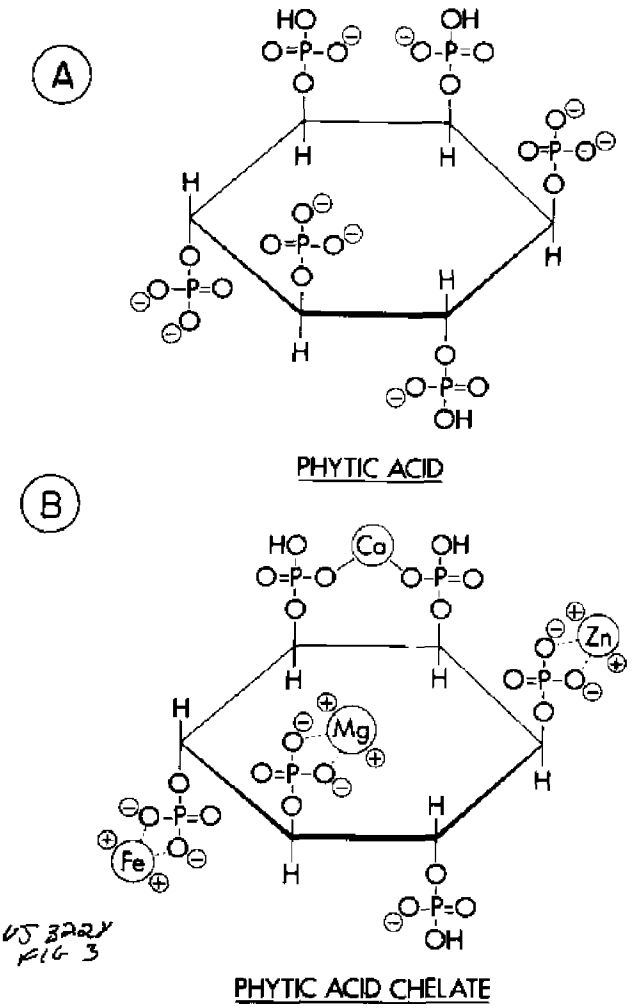

FIG. 3. Structures of phytic acid (A) and A phytic acid chelate (B) at neutral pH. Structures (A) and (B) were drawn by Weingartner and Erdman (22) and are conjectural.

OCCURRENCE

Phytic acid concentration in whole grain cereals such as corn, wheat and rice is ca. 1.0% (d.b.) (Table 1), while defatted and dehulled oilseed meals such as soy, peanut, and sesame meals contain 1.5% or more of the compound (Table II). In most seed types the phytic acid is associated with specific components within the seed and can be preferentially extracted with those components. For example, the endosperm of wheat and rice kernels are almost devoid of phytate as it is concentrated in the germ and aleurone layers of cells of the kernel and in the bran or hull. Corn differs from most cereals as almost 90% of phytic acid is concentrated in the germ portion of the kernel (27).

In oilseeds, which contain little or no endosperm, the phytates are distributed throughout the kernel located within subcellular inclusions called aleurone grains or protein bodies (12). Phytate in peanuts, cottonseed, hempseed, and sunflower seeds is concentrated in substructures – crystaloids or globoids – within the protein body membrane (28). Lui and Altschul (29) isolated globoids from cottonseed aleurone grains and found them to be low in protein, fat and carbohydrate content but high in phytic acid (60%) and metals (10%). In contrast to other oilseeds, soybean phytic acid, although concentrated within protein bodies, appears to have no specific site of localization (30).

TABLE I

Phytic Acid Concentration in Morphological Components of Cereals[a]

Sample	Phytic acid[b] %	Distribution[c] %
Corn, Hybrid	0.89	—
Germ	6.38	88.0
Endosperm	0.04	3.2
Hull	0.07	0.4
Wheat, Soft	1.13	—
Germ	3.90	12.9
Endosperm	< 0.01	2.2
Aleurone	4.11	87.1
Hull	None detected	None detected
Rice, Brown	0.89	—
Germ	3.48	7.6
Endosperm	0.01	1.2
Pericarp	3.37	80.0

[a]Calculated from data of O'Dell et al. (27), assuming 28.2% phosphorus in phytic acid.

[b]Based on air dry weight.

[c]Percentage of the element in the component part.

Isolation of subcellular fractions of oilseeds can result in quite variable concentrations of phytic acid. Dieckert et al. (31) isolated two protein-rich fractions from peanuts and found one to contain 0.5% phytic acid while the other, an aleurone grain-containing, protein-rich fraction, had 5.7% phytic acid. Some rapeseed concentrates have been reported to contain over 7.0% of the chelator (32). Current literature suggests that wheat and oilseed phytate occurs primarily as potassium-magnesium salts (33,34).

NUTRITIONAL IMPLICATIONS

Animal experiments have suggested that phytic acid in plant foods binds dietary essential minerals making them unavailable for absorption. In vitro studies have shown that many phytic acid mineral complexes are insoluble at intestinal pH and are though to be biologically unavailable for absorption (10,35-37). The formation of these complexes is pH-dependent. When two or more cations are present, one may find a synergistic increase in precipitation of phytate salts. This phenomenon has been demonstrated in vitro with zinc and calcium, and zinc and copper (1,10). Mineral interactions in situ are not clear and are complicated by protein-mineral-phytate interactions, for example.

Poor mineral utilization from high phytate foods cannot be directly attributed to phytate binding since fiber and other constituents of these foods may play major roles. Some researchers have added pure phytate to food systems and have found reduced mineral uptake. This type of investigation reveals little insight into the chemical binding of naturally occurring phytic acid, especially when excessive levels of sodium phytate are added to food systems (4). However, many other studies strongly suggest a correlation of phytic acid and mineral malabsorption. One cannot directly equate the phytate concentration with mineral bioavailability in phytate-containing food, since other factors such as the food processing history are very important (38,39). But, removal of phytate seems to improve mineral bioavailability. Recently, for example, Ellis and Morris (40) reported that incubation of high phytate, high fiber wheat bran to hydrolyze phytate resulted in higher utilization of wheat bran iron and zinc.

Selected research reports dealing with the possible action of phytic acid in reduction of the bioavailability of calcium, magnesium, zinc, iron and phosphorus from oilseed products are presented below. Except for a few recent studies with rapeseed, most published research dealing with the bioavailability of these minerals from oilseeds has been carried out with soybean products. Results from soy studies cannot be directly applied to other oilseeds due to differences in localization of phytic acid within oilseed kernels (30).

TABLE II

Phytic Acid Concentration in Various Oilseed Products[a]

Sample	Phytic acid[b] %
Soybean meal, full-fat or defatted and dehulled	1.4-1.6
Soybean protein isolate, concentrate	1.6-2.2
Peanut meal, defatted and dehulled	1.7
Sesame meal, dehulled	3.6
Sesame meal, defatted and dehulled	5.2
Rapeseed protein concentrate	5.3-7.5
Cottonseed flour, glanded	2.9
Cottonseed Flour, glandless	4.8
Cottonseed globoids	60.0

[a]See refs. 35, 31, 76, 32, 29, 34, 77 plus information from the author's lab.

[b]Data in most cases is calculated on the dry basis and assumes 28.2% phosphorus in phytic acid.

CALCIUM

In a series of papers in the 1920s, Mellanby reported anticalcifying and rachitogenic properties of certain cereals fed to dogs. Mellanby (41) found that the rickets-producing effects of oats could be reduced by boiling the oats with a mineral acid or by subjecting them to a malting process. By 1949, Mellanby (42) demonstrated that phytate addition to dog diets reduced calcium absorption and subsequently indiced rickets. A few years later Walker (43) warned that low dietary calcium and/or vitamin D (or sunlight) were important in the etiology of rickets and that the incrimination of phytic acid was far from conclusive. Thus began the debate over phytic acid's causative actions in mineral malnutrition.

Walker and associates (44) reported that retention of dietary calcium and magnesium improved in human subjects after short periods on high phytate diets. They felt that gradual adaptation takes place as the body increases its absorption efficiency. Reinhold et al. (45) failed to confirm human adaptation, as men fed both naturally occurring and purified phytate for 60 days maintained negative calcium balance for the entire period.

The calcium content of oilseeds is not high, which may explain why few studies have been reported in the literature concerned with the bioavailability of calcium from oilseeds. Rats, for example, fed diets containing 40% soy flour will derive only 10% of their calcium requirement from the soy.

Experiments have been recently conducted at the University of Illinois (46) to test the effects of the presence of various types of soy products in rat diets upon the bioavailabity of calcium added in incremental levels to diets as calcium carbonate. A slope-ratio assay procedure (47) was used to compare the regression of femur calcium upon total calcium added to soy or casein diets. Male albino rats were fed ad libitum 18% protein diets from full-fat soy flour, freeze-dried soy beverage, a commercial soy concentrate or casein for about 4 weeks. The results clearly indicated that the bioavailability of calcium added as calcium carbonate to any of the three soy products was the same as when added to casein diets. These results suggest that calcium fortification of oilseed products will result in good utilization of calcium. However, as pointed out in previous publications (1,3,10,48), calcium addition may aggravate zinc, magnesium or copper utilization from oilseed products which are high in phytic acid.

MAGNESIUM

Roberts and Yudkin (49) reported that magnesium deficiency symptoms could be aggrevated by addition of sodium phytate to casein-based diets. Forbes (50) found that magnesium absorption, but not balance, was reduced in young rats fed isolated soy protein diets in comparison to egg white protein diets. Recent studies from Forbes' laboratory (46) evaluated the bioavailability of magnesium from three standard soy products as compared to $MgCO_3$ added to casein diets. Standard additions of magnesium to rat diets resulted in linear increases in serum and tibia magnesium. Regression of these criteria on dietary magnesium from soy flour and soy beverage showed full bioavailability of the mineral while magnesium from a commercial soy concentrate was ca. 80% as available as that from the inorganic source. These results and others reported by Lo et al. (51) demonstrate that the bioavailability of magnesium from or added to soy products is very good.

IRON

In some studies, foodstuffs containing phytate have been shown to be particularly inhibitory to iron absorption (52-55), while in other studies high levels of phytic acid were reported to be without an effect (56,57). Iron bioavailability from soybean proteins has been reported to range from 28.5-80% (of inorganic iron) in rat studies (57-59). The most recent work of Steinke and Hopkins (59) compared hemoglobin repletion in rats fed one of three soy protein isolates with rats fed ferrous sulfate. They found a mean relative iron bioavailability of 61% for soy isolates and further reported that inorganic iron added to diets containing isolated soybean protein had bioavailabilities similar to that of the iron present in the soybean. These results, coupled with the relatively high iron content of many soybean products, suggest that the iron supplied by these products can be of significant value for the human diet (59).

ZINC

Perhaps the greatest impact of phytic acid on human nutrition is its reduction of zinc bioavilability. Numerous studies have implicated phytic acid as a causitive factor in poor zinc absorption from plant foods (2–4). In the 1960s, workers in the laboratories of O'Dell (3) and Forbes (3,50,60) established a general inverse relationship of phytic acid content of animal diets and zinc bioavailability from those diets. Maddaiah et al. (36) studied zinc deficiency in the chick and reported that at physiological pH zinc formed the most stable (insoluble) complex with phytic acid. The decreasing order of stability of phytate-mineral complexes was reported as zinc, copper, nickel, cobalt, magnesium and calcium. Vohra et al. (37) investigated the stability of phytic acid-metal complexes at pH 7.4 by titration methods and reported the decreasing order of stability as copper, zinc, nickel, cobalt, iron, and calcium. Although calcium has the lowest binding affinity, addition of this cation to diets containing phytic acid was shown to reduce zinc and magnesium absorption (3,48).

Most recently, Forbes and Parker (61) and Erdman et al. (46) utilized a slope ratio assay method to test the bioavailability of zinc using growth as well as log of femur zinc of young rats as a criterion of response to increased dietary zinc concentrations. It was found that: (a) zinc was poorly utilized from soy products as compared to other studied minerals, (b) zinc bioavailability from soy products seems to vary from product to product, i.e., zinc was better utilized from a full fat soy flour than from a soy concentrate, and (c) usually, the presence of soy products in the rat's diet had little effect upon the bioavailability of added inorganic zinc carbonate. Momcilovic and Shah (62) concluded from work with rapeseed protein concentrates (some of these concentrates contain in excess of 7.5% phytic acid [32] that the poor availability of zinc from diets containing the rapeseed protein concentrates was the major rate-limiting factor for growth and development of young rats. Furthermore, they (63) fed a milk-based infant formula and a soy-based formula to young rats and found zinc utilization to be poorer with the latter formula.

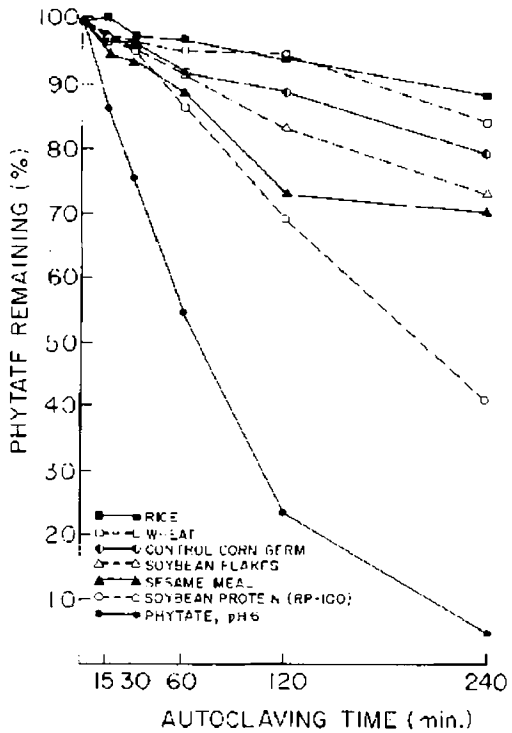

FIG. 4. Rate of loss of "phytate" during autoclaving inositol hexaphosphate and moist slurries of various natural products at 115 C. Source: de Boland et al. (35).

The role of fiber in decreasing zinc utilization should not be overlooked (9). A current in vitro study by Reinhold's group (6) suggests that some dietary fiber components may be more effective in mineral binding than phytic acid. However, Weingartner, et al. (64) have found the zinc and calcium bioavailabilities were not affected by the addition of soybean hulls (that contain over 50% fiber) to dehulled soy flour-based diets for rats.

Rackis and his colleagues (38,39) point out that zinc utilization from certain soy isolates has been shown to be particularly low as compared to zinc utilization from soybean meals. They suggest that the difference in zinc bioavailability is most likely due to the varying food processing conditions used in manufacturing the soy protein products. They feel that the type of phytate-protein-mineral complexes formed during processing, rather than the specific phytate contents, are responsible for different absorption capacity. "Endogenous zinc carriers" (65) (see recent review of regulation of zinc absorption. Cousins [66]) must compete with phytate-mineral and phytate-protein-mineral complexes within the lumen of the intestine to "solubilize" zinc for absorption. Research is needed to identify the process steps that affect formation of phytate complexes (11,38,39).

PHOSPHORUS

In mature cereal grains, 60-80% of the total phosphorus is tied up in phytic acid (23). Soybeans contain almost

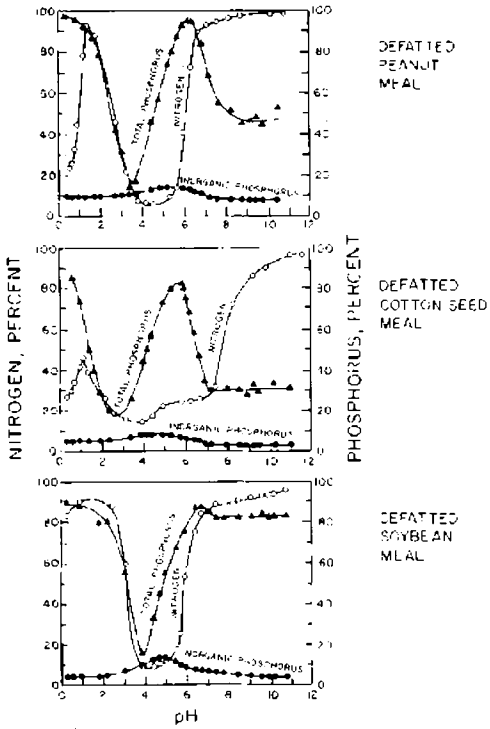

FIG. 5. Solubility of nitrogen and phosphorus compounds of defatted peanut, cottonseed, and soybean meals at various pHs. The percentage of the total nitrogen and phosphorus of solvent-extracted oilseed meals which are soluble in HCl and NaOH solutions at different pH values. Source: Fontaine et al. (74).

twice as much phosphorus as cereals, but 50-60% is phytic acid phosphorus (67). Bioavailability of the phosphorus for animals seems to depend upon the level of phytase activity in the intestinal tracts of the specific species (13,67). Insufficient bioavailability studies with humans have been published to predict phytate phosphorus availability. However, one might suspect that it is rather poor since monogastric animals have little or no phytase activity (8). In this country adults generally consume excessive levels of phosphorus. Therefore, one can assume that phosphorus bioavailability is of little consequence for this group. On the other hand, phosphorus bioavailability for U.S. children consuming soy-based infant formulas is of considerable importance. Proper bone development of infants requires adequate levels of available phosphorus.

OTHER MINERALS

Trace minerals, such as copper, manganese, molybdenum, and cobalt, may also be affected by oilseed phytates. In addition, imbalances of minerals may occur due to preferential binding of certain minerals (13). For example, Klevay (68) suggests that the strong binding of zinc to dietary phytic acid at intestinal pHs to form insoluble complexes would favor dietary copper absorption and thus change the zinc-copper ratio. According to Klevay, this change would be beneficial from the standpoint of serum cholesterol and coronary heart disease; however, it would be harmful from the standpoint of zinc metabolism.

METHODS OF REMOVAL OF PHYTATE AND REDUCING PHYTATE-MINERAL BINDING

Research papers discussed in the previous sections suggest that phytic acid is at least partially responsible for reduced bioavailability of certain minerals from high phytate foods. Since monogastric animals such as man have little or no intestinal phytase activity, it would be advantageous to develop methods to either remove phytic acid from cereals and oilseeds or to reduce its mineral-binding capacity. Figure 4 demonstrates the impracticality of using heat to cleave minerals from phytate. de Boland et al. (35) clearly show that 30 minutes autoclaving reduces phytate content of cereal and oilseed products by less than 10%.

Tempering or soaking oilseeds is unlikely to reduce phytate appreciably due to rather low endogenous phytase contents of oilseeds. However, yeast will cleave phosphates from phytic acid during fermentation of legumes. Ranhotra et al. (69) demonstrated that all of the phytate in wheat bread and more than three-fourths of that in soy-fortified wheat flours (soy, 10%, wheat, 90%) was hydrolyzed during the process of breadmaking, apparently due to phytases in the wheat and/or yeast.

Several groups of workers have attempted to separate phytate from soybeans utilizing the differential solubilities of phytate and soybean protein (70-73). From Figure 5 it is apparent that the solubility of phosphorus compounds does not correspond to that of nitrogenous constituents of any of three oilseeds, except in the general pH region of 1.5 to 3.5. Fontaine and coworkers (74) suggest that up to about pH 3.5 most of the phosphorus apparently is combined with proteins. However, as the proteins approach and pass through their isoelectric points, the protein-phytic acid complexes dissociate. Above pH 7, defatted soybean meal differs from peanut and cottonseed meals as there is little decrease in solubility of phosphorus compounds. This may be due to reassociation of phosphorus compounds with protein, as suggested by Rackis et al. (38). The percentage of total phosphorus in the three meals found to be in inorganic form was generally less than 10%. Some increase of inorganic phosphorus is evident in the area of pH 5. This increase is undoubtedly due to the enzymatic activity of native phytases, since the time elapsing between the preparation of extract and analysis was ca. 4 hr (74).

Figure 5 predicts that one could separate phytate from soybean products by solubilizing the chelator at pH 5.0 to 5.5, followed by centrifugation or ultrafiltration. Forc et al. (72) and Okubo et al. (70) describe procedures for phytate removal in this pH range. In addition, these same groups were able to remove phytic acid at pH 3.0 to 4.0 by adding calcium chloride to dissociate the phytate-protein complexes. Since calcium ions mediate phytate-protein binding above the isoelectric point (75), Okubo et al. (70) were able to facilitate dissociation of phytate-protein complexes at pH 8.5 by chelating free cations with EDTA addition. Ultrafiltration was then used to remove phytate. Hartman (73) has described a phytate removal process at pH 11.6.

Martinez (12) has reviewed the current knowledge of food processing effects upon phytate destruction or removal from plant foods. She points out that the manner in which oilseeds are fractionated and processed directly affects the disruption of membranes surrounding aleurone protein bodies and membranes surrounding inclusions containing phytic acid. Formation of insoluble phytate complexes with protein or other food components during food processing then depends upon the extraction pH, and the presence of minerals or other chelators. The extractability of phytate phosphorus from individual oilseeds varies due to the morphological differences between seed types. Therefore, one cannot apply similar process techniques to all types of oilseeds.

Obviously, more basis research is needed to identify the important mineral binders and to study the mineral chelation during processing of various oilseeds. Then, economically feasible technology must be developed to eliminate the chelators or the chelation. In addition, we must ascertain the effects of mineral addition (fortification) upon the

bioavailability of both the fortified and the native minerals. Such questions must be addressed before true assessment of the overall nutritional potential of oilseeds can be made.

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ADDITION

For the reader's benefit, the following references, not referred to in the text, have been published after the original paper was submitted.

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