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REVIEWS

Phytate: Its Chemistry, Occurrence, Food Interactions, Nutritional Significance, and Methods of Analysis

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This review provides a current summary of the literature concerning various aspects of phytate. These include data relative to its chemical structure, its occurrence in numerous cereals and legumes, the role of phytase, and the influence of food-processing conditions on phytate/phytase activity. In addition, the nutritional significance of phytate with regard to mineral binding abilities and methods commonly used for the analysis of phytate are also discussed.

Phytates represent a complex class of naturally occurring compounds that can significantly influence the functional and nutritional properties of foods. Although their presence has been known for over a century, their role is not completely understood and thus represents an area of active research.

A brief review has appeared (Oberleas, 1973), but on the basis of the extent and importance of the literature, an attempt will be made in this review to expand upon the chemical, functional, and nutritional implications of phytates in our food supply.

CHEMISTRY

Numerous polyphosphorylated inositols can be found in nature, and depending on the complex formed, a wide variety of compounds can exist. This has perhaps lead to some of the confusion in terminology concerning the nomenclature of these compounds with terms such as phytin, phytate, phytates, and phytic acid being prevalent in the literature. It is now generally belived that the compound phytic acid can be commonly called *myo*-inositol hexaphosphoric acid or, scientifically, 1,2,3,4,5,6-hexakis(dihydrogen phosphate) *myo*-inositol (IUPAC–IUB, 1968). The term phytin implies a calcium–magnesium salt of phytic acid, whereas phytate would mean the mono to dodeca anion of phytic acid. However, commercially available phytic acid or phytate often contains lower phosphate derivatives than hexaphosphate (Cosgrove, 1963), which some investigators include in the term phytates. Angyal and Russell (1969) have questioned whether the presence of these lower esters results from steps in the biosynthesis of the hexaphosphate form or they are actually chemical degradation products due to the isolation procedure. It has been shown by Cosgrove (1966) that phytic acid can be dephosphorylated by phosphatase enzymes commonly called phytases and also by heating in acid or alkaline solutions.

Pfeffer (1872) was apparently the first to report that globules composed of calcium and magnesium salt and an organic phosphate were present in aleurone grains. Winterstein (1879) extracted a similar substance from mustard seeds and upon hydrolysis with hydrochloric acid reported that *myo*-inositol and orthophosphoric acid were the by-products.

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Adding to the structural confusion is the fact that nine sterisometric inositols are possible (*cis*-inositol, *epi*-inositol, *allo*-inositol, *neo*-inositol, *myo*-inositol, *muco*-inositol, *chiro*-inositol and its enantiomer, and *scyllo*-inositol). To date, only the myo form has been isolated from plants, while *neo*-, *chiro*-, and *scyllo*-inositol hexaphosphates have been identified in soils (Cosgrove, 1966). Oberleas (1973) postulated that these latter forms represent the influence of either microorganisms or different soil types.

Also, the hexaphosphate forms have not been found in animal tissues whereas mono-, di-, and triesters are associated with complex lipids in animals (Burns and Conney, 1960). Johnson and Tate (1969a) reported on the structures of six *myo*-inositol pentaphosphates, one of which they reported to be present in chicken blood (Johnson and Tate, 1969b). In addition, *myo*-inositol pentaphosphates have been identified from the chemical and enzymic hydrolysis of cereal grain phytic acid (Tomlinson and Ballou, 1962; Cosgrove, 1963; Angyal and Russell, 1968) and microorganisms (Greaves et al., 1969; Cosgrove 1969, 1970; Cosgrove et al., 1970). Johnson and Tate (1969a) also concluded that at least two phytases exist in biological systems, with one removing the 1-phosphate and the other the 6-phosphate from phytic acid.

Johnson and Tate (1969b) have supplied convincing evidence, through the use of ^{31}P nuclear magnetic resonance, to support the phytic acid structure, proposed by Anderson (1914a), who suggested a symmetrical hexaorthophosphate arrangement in contrast to the Neuberg (1908) asymmetrical hydrated tripyrophosphate structure. It should be noted that convincing evidence also has been presented indicating that three strongly bound water molecules are part of phytic acid (Neuberg structure) by Brown et al. (1961), and thus Erdman (1979) has recently postulated that both structural forms may exist. In addition, Weingartner and Erdman (1978) have suggested a partially dissociated Anderson-type structure which could occur at a neutral pH. However, Erdman (1979) does acknowledge that most evidence points toward the Anderson structure.

The conformation of the phytic acid molecule is still in question. Johnson and Tate (1969b) suggested that the 2-phosphate is in the axial position while the phosphate groups on carbons 1 and 3–6 are equatorial, whereas Blank et al. (1971), through X-ray analysis, concluded that the 2-phosphate group is equatorial and the others axial. It should be noted that the study of Johnson and Tate (1969b) utilized a dilute solution, whereas Blank et al. (1971) used a single sodium phytate crystal. The fact that a number of different crystalline forms of sodium phytate are known and that they differ in their degree of hydration (Posternak, 1921; Brown et al., 1961; Johnson and Tate, 1969b) can perhaps be used to explain this apparent discrepancy. Costello et al. (1976) have recently demonstrated the effect of pH on phytic acid configuration. Thus, it would appear that phytate stereochemistry can be quite variable, which makes the compound quite reactive during the processing and consumption of food.

OCCURRENCE

Phytates can be found in a wide variety of food as was demonstrated in an early study of Averill and King (1926), who reported a wide range of phytate levels as influenced by variety and byproduct of numerous cereals and nuts. Phytate levels in numerous feed ingredients have also been measured (Nelson et al., 1968a). Phytates have been found in cereal grains and legumes up to a level of approximately 5% by weight (de Boland et al., 1975), and perhaps at this point it would be advisable to attempt to group the extensive literature relative to phytate occurrences in these products.

Barley. Utilizing scanning electron microscopy, Pomeranz (1973) observed that in the case of barley, phytate is in the form of potassium and magnesium salts instead of the calcium–magnesium complex normally thought to be present in most other cereals. Phytic acid levels in 18 varieties of barley were found to range from 0.97 to 1.08% dry weight (Lolas et al., 1976).

Dry Beans. Makower (1969) reported that mature dry pinto beans contained approximately 1% phytic acid whereas immature beans contained about 0.13%. In addition, low levels of phytic acid were found in the pod at all stages of maturity. Makower (1970) later reported on the effectiveness of four different phytic acid analytical procedures as influenced by pinto bean maturity.

Walker (1974) reported that embryo development to maturity in *Phaseolus vulgaris* requires approximately 36 days. During that time phytic acid was found after 12 days but did not begin to accumulate rapidly until after day 24 and slowed after 30 days. Approximately 90% of the phytic acid developed between days 24 and 30. During germination 90% of the phytic acid was lost by day 10.

Lolas and Markakis (1975) measured the phytic acid level of 50 cultivated varieties of *P. vulgaris* grown over a 2-year period and found a range of 0.54–1.58%. They also found a high degree of correlation between total phosphorus content and phytic acid level. In addition, a protein–phytate complex was isolated, and it was noted that over 99% of the total phytic acid was in a water-soluble form which could serve as a means of removing or lowering phytic acid levels in this product.

Corn. Anderson (1914c) was among the first to identify phytate in corn. Later, DeTurk et al. (1933) followed phytate levels in corn from pollination to maturity and observed that phytate was not present in the leaves, stems, tassels, or cobs of the plant and that phytate began to increase in the kernals approximately 3 weeks after pollination and increased to maturity. Data of a similar nature were reported by Earley and DeTurk (1944).

O'Dell et al. (1972b) demonstrated that in corn approximately 90% of the phytate is concentrated in the germ portion as compared to the endosperm and hull portions. A similar observation was made earlier by Hamilton et al. (1951). Thus, foods that incorporate corn germ can be unusually high in phytates. No significant differences in phytate content in normal and high-lysine corn has been found (O'Dell et al., 1972b; de Boland et al., 1975; O'Dell and de Boland, 1976).

Cottonseed. Anderson (1914a) identified phytate in cottonseed meal. Ergle and Guinn (1959), in working with germinating cottonseed, noted the dephosphorylation of phytate resulted in the accumulation of inorganic phosphorus. Phytate hydrolysis was found to be increased by light. Varietal differences were also found.

Wozenski and Woodburn (1975) measured the phytic acid level in four food-grade cottonseed products and found 2.86 g/100 g in a defatted glanded flour, 4.29 g/100 g in a glandless flour, 3.35 g/100 g in an air-classified glandless flour, and 2.49 g/100 g in toasted seed kernels. Interestingly, the phytate level in the glandless products was significantly higher than that in the glanded.

Oats. Anderson (1914b) had confirmed that oats contained phytate. Ashton and Williams (1958) found that phytate phosphorus is gradually broken down into inorganic phosphorus during the germination of oats and that no phytate phosphorus was present after 2 weeks of germination. During panicle emergence and up to the milk-

ripe stage, they found no phytate in maturing oats; however, at maturity approximately 60% of the phosphorus was in the form of phytate. They also reported significant differences in phytate levels as influenced by oat variety, thus indicating that genetics may be used as a tool to influence phytate levels.

Hall and Hodges (1966) were following phosphorus metabolism during the germination of oats and reported that during the first 8 days of germination phytic acid phosphorus was converted entirely into inorganic phosphorus and appeared in the roots and shoots.

Lolas et al. (1976) evaluated phytic acid levels in 19 oat varieties and found a range of 0.84–1.01% based on dry weight and proposed that total phosphorus measurement could be used to estimate phytic acid content. Similar conclusions have been reached (Miller et al., 1980a).

Miller et al. (1980a) evaluated phytic acid levels in oats as influenced by variety, year of growth, and location of growth. All of the varieties (four) were different when averaged across years (four) and locations (three). Yearly differences in phytic acid levels were significant at some locations. Also, varieties ranked the same in phytic acid levels for overall years and locations, thus indicating that phytic acid production is affected by environment. Miller et al. (1980b) also observed that oat phytic acid concentration was positively correlated to available soil phosphorus levels.

Peanut. Phytate in peanuts is apparently concentrated in substructures within the protein body membrane (Saio et al., 1977). However, with peanuts Dieckert et al. (1962) isolated one protein-rich fraction that contained 0.5% phytic acid while an aleurone grain-containing protein-rich fraction contained 5.7%.

Peas. In developing an analytical procedure for phytate phosphorus, Holt (1955) used dried peas as a model. Phosphate levels during the development of peas were measured by Rowan and Turner (1957) and Chen and Pan (1977). In the latter study, between 50 and 60% of the phytate was still present 5 days after germination. This and similar observations on residual phytate levels during germination should be considered when various types of sprouted seeds are consumed. Early research (Mattson, 1946) indicated that the texture of cooked peas was dependent on their phytate content; however, Crean and Haisman (1963) concluded that phytate levels had little influence on pea texture.

Rapeseed. Numerous rapeseed products have been shown to have relatively high levels of phytic acid. Rapeseed meal has been found to contain approximately 3–5% (Nwokolo and Bragg, 1977; Uppstrom and Svensson, 1980), while rapeseed protein concentrate reportedly has even higher levels (Shah et al., 1976).

Rice. Asada and Kasai (1962) reported that during the early stage of rice ripening a major portion of the *myo*-inositol was in the free state but at the end of the ripening period most of the *myo*-inositol was in the phosphate ester form which represented approximately 80% of the total phosphorus in the product. Free *myo*-inositol and *myo*-inositol phosphate was found in the grains, leaves and stems, and roots of rice, but the low levels in the latter two portions compared to the grain level indicated that biosynthesis occurred in the grains themselves from sugars. A later study by Asada et al. (1969) demonstrated that the amount of phytic acid formed in rice grains is directly affected by the amount of phosphate available to the plant, and they postulated that excess phosphate could be stored in the form of phytic acid or phytate. Thus, excessive phosphate fertilization could result in high phytate levels.

Kennedy and Schelstraete (1975) reported that phytic acid was primarily found in the outer layers of rice grain. Specifically, 2% of the outside kernal was found to contain 23 times more phytic acid than the intact kernal, and removal of the outer 13% of the kernal resulted in an endosperm that contained no detectable phytic acid. Thus, if specifically concerned about phytic acid levels in rice, milling can be used to decrease its level. de Boland et al. (1975) reported that brown rice contained 0.89% phytic acid whereas the germ had 3.48% and the pericap had 3.37% with the endosperm having 0.01% based on air dry weight (O'Dell et al., 1972b) as calculated by Erdman (1979).

Toma and Tabekhia (1979) recently reported that the water quality used during the cooking of rice can significantly influence phytic acid levels. Cooking for 10 min in domestic tap water resulted in no significant loss of phytic acid; however, when distilled, deionized water was utilized, phytic acid content was reduced by approximately two-thirds. The ability of phytic acid to form salt complexes was cited as the reason for this difference.

Sesame. Sesame seeds have been reported to contain the highest levels of phytate (5.18%) found in nature (de Boland et al., 1975). Apparently, however, its level can vary dramatically since a later study by the same group (O'Dell and de Boland, 1976) reported a level of 1.44%.

Soy. Phytates in soy appear to be unique in that although associated with protein bodies, they appear to have no specific site of localization (Tombs, 1967). Lolas et al. (1976) reported that the phytic acid content of 15 soybean varieties ranged from 1.00 to 1.47% dry weight which represented between 51.4 and 57.1% of the total phosphorus. The phytic acid content in several commercially available soy products was reported by de Boland et al. (1975). Soy meal had a level of 1.42%, flakes, 1.52, and isolate, 1.52%. One would have perhaps anticipated that since phytate is apparently associated with protein, phytate levels should increase with increasing protein content. For some reason the data do not support this theory. Another study of O'Dell and de Boland (1976), using experimentally prepared soy flakes, found them to contain 0.43% phytate.

Sunflower. Sobolev and Rodionova (1966) followed phytate synthesis during the ripening of sunflower seeds and concluded that its biosynthesis is not associated with the utilization of hexose phosphates or glycolysis products but is due to the stepwise phosphorylation of inositol.

Saio et al. (1977) found that certain isolated protein bodies from sunflower seed were rich in phosphorus, which probably represented phytate storage sites since similar observations have been noted with cottonseed (Lui and Altschul, 1967).

Wheat. On the basis of their observations, Jennings and Morton (1963a) suggested that phosphate–protein complexes could serve as intermediates in the formation of storage proteins. In a later study (Jennings and Morton, 1963b), they reported that initiation of rapid phytic acid synthesis could be correlated to the time of restriction of supply of water to the endosperm during maturation. Williams (1970) also investigated the effect of water stress on phytic acid formation in maturing wheat and found similar results.

O'Dell et al. (1972b) found a level of 0.32% phytate in whole kernal wheat with approximately 87% of it being associated with the aleurone layer, 13% in the germ, 2% in the endosperm, and none in the hull portion.

In evaluating 11 wheat varieties, Nahapetian and Bassiri (1975) observed that major phytic acid synthesis during maturation occurred during the chlorophyll destruction

stage and thus suggested that chlorophyll levels can be used to determine maturation.

Morris and Ellis (1976) have reported that most of the phytate associated with wheat bran is in the form of monoferric phytate which in turn is probably bound to cationic sites of proteins or other cellular components.

In evaluating 38 wheat varieties, Lolas et al. (1976) found a phytic acid range of 0.62–1.35% dry weight in whole kernels whereas the bran portion had phytic acid levels ranging from 4.59 to 5.52%, demonstrating that foods containing added wheat bran could have unexpectedly high phytate levels.

Miscellaneous Foods. Although this portion is far from complete, other products that have been shown to contain phytates include crested wheatgrass (Wilson and Harris, 1966), potatoes (Samotus and Schwimmer, 1962), the aquatic angiosperm *Wolffiella floridana* (Roberts and Loewus, 1968), and the Bengal gram seed (Naik and Narayana, 1959; Verma and Lal, 1966; Reddy and Salunkhe, 1981). In addition, Oberleas (1973) reported that lettuce, onions, mushrooms, celery, and spinach do not contain phytates, while green beans, carrots, and broccoli have only trace amounts. In contrast, potatoes, sweet potatoes, and artichokes have moderate amounts, while cereals, nuts, and legumes contain even higher levels. He also reported that certain fruits (citrus, apples, pineapple, bananas, and prunes) contain no phytates while other (blackberries, strawberries, and figs) contain small to moderate amounts.

PHYTASE

Phytase was one of the first enzymes to be described that liberates inorganic phosphate from organic phosphorus containing compounds (Suzuki et al., 1907) and as such has widespread distribution in plant and animal tissues (Peers, 1953; Kindl, 1969) and numerous types of microorganisms (Nelson et al., 1968c; Shieh and Ware, 1968; Tanner, 1969).

Although phytase is present in mature seeds, it appears to have little effect on phytate in dry or dormant seed. However, Glass and Geddes (1959) found an increased level of inorganic phosphorus along with lower phytate levels in wheat stored under elevated temperature and moisture conditions. Thus, they suggested that the level of inorganic phosphorus could be used to indicate adverse storage of wheat.

Peers (1953) extensively investigated phytase activity in wheat and concluded that hard wheats had higher levels than soft but that the variation in activity among the species was not large. He also reported that phytase was mainly found in the aleurone (39.5%) portion, followed by the endosperm (34.1%) and scutellum (15.3%), and that the optimum temperature for hydrolytic activity was 55 °C.

Consumption of whole wheat based breads that have been made with little or no fermentation has caused concern because of their phytate levels (Kouhestani et al., 1969; Reinhold, 1971, 1972, 1975; Ter-Sarkissian et al., 1974; Reinhold et al., 1976). Yeast or sourdough fermentation of doughs has been shown to lower phytate levels by one-third to half (Reinhold, 1972). Earlier, Mellanby (1944) had demonstrated that fermentation time, temperature, pH, and humidity could all significantly influence phytase activity. Reinhold (1975) reported that phytate loss due to the action of phytase was rapid in fermented bread made from 75–90% extraction wheat but was slow for 95–100% extraction products. de Lange et al. (1961) reported that phytate is completely hydrolyzed in low extraction flours.

Recently, Harland and Harland (1980) reported on the phytate reduction in rye, white, and whole wheat breads as influenced by the amount of yeast used in fermentation as well as by fermentation time. Increasing the amount of yeast and time of fermentation did significantly reduce phytate levels in all products, but one has to question the practical application of this approach. They reported that normally prepared whole wheat bread had a phytate level of 0.56% dry weight, rye at 0.41%, and white bread at 0.03%. Rye apparently contains a more active phytase than most other cereals (Hoff-Jorgensen et al., 1946).

The state of phosphorus, including phytate phosphorus, during various stages of bread making has also been evaluated by Tangkongchitr et al. (1981a,b). They reported that in whole wheat pup loaves the loss of phytate phosphorus after fermentation, proofing, and baking was 16, 19, and 22%, respectively (Tangkongchitr et al., 1981b). Phytate phosphorus was found to be quantitatively converted to inorganic phosphorus. Evaluation of commercial whole grain breads had phytate phosphorus levels ranging from 218 to 808 mg/lb, whereas white bread ranged from 87 to 173 mg/lb (Tangkongchitr et al., 1981b). They postulated that the primary factor limiting phytate destruction during bread making is the relative insolubility of magnesium phytate in the dough. In contrast to the conclusions of Harland and Harland (1980), Tangkongchitr et al. (1981b) did not find a significant loss of phytate with higher yeast levels. They (Tangkongchitr et al., 1981b) postulated that the change in dough pH is the primary means by which yeast fermentation enhances phytate loss and since the addition of 2 or 4% yeast resulted in no significant change in pH during fermentation, phytate levels were not influenced.

Compared to wheat flour, wheat protein concentrate has been reported to have a phytic acid level approximately 20 times higher (Ranhotra, 1972), and thus the inclusion of this product into bakery items can present unique phytate-related problems in spite of the fact that a high level of phytase activity is associated with wheat protein concentrate. Apparently the phytase is not fully active due to a nonoptimum pH situation when high levels of wheat protein concentrate are utilized. Calcium additions have been shown to inhibit phytate hydrolysis in bread making (Ranhotra, 1972), and thus additives that can be high in calcium, such as certain whey products, can prevent phytase activity (Ranhotra, 1973).

The potential for soy phytate to undergo enzymic hydrolysis during bread baking was investigated by Ranhotra et al. (1974a). The phytase activity of numerous commercially available soy products was also evaluated, and all products were found to have little activity. Similar observations were earlier noted by Mollgaard et al. (1947). The addition of 10% soy product to the bread formulation resulted in phytate hydrolysis in excess of 80% based upon the initial phytic acid levels of approximately 300 mg/loaf, which was approximately twice as high as the no-soy control of 134 mg/loaf. In contrast, hydrolysis of a whey–soy blend product was only 22%, probably due to high residual levels of calcium in the product.

Wozenski and Woodburn (1975) measured the phytase activity associated with several cottonseed products, and all but one did possess some activity. They postulated that phytase activity was probably lowered or lost during heat processing.

The phytase activity associated with beans and peas has been investigated. For example, Gibbins and Norris (1963) reported that phytase isolated from *P. vulgaris* had an optimum pH for activity of 5.2. Kon et al. (1973) at-

tempted to reduce the phytate content of *P. vulgaris* by adding wheat phytase, followed by incubation before and after cooking. However, only up to 15% hydrolysis was obtained. Phytase activity during the germination of *P. vulgaris* was followed by Walker (1974). He found no activity during embryogeny, but a rapid rise in activity appeared after day 2 of germination. Similar results were obtained with peas (Chen and Pan, 1977) and mung beans (Biswas and Biswas, 1965).

Wang et al. (1980) evaluated the ability of various molds used in the production of oriental-based fermented foods such as sufu, tempeh, lao-chao, soy sauce, and miso to produce phytase. All molds evaluated, except *Mucor dispersus* NRRL 3103, produced phytase. One of the most active phytase-producing molds was *Aspergillus oxyzae* NRRL 1988. Its optimum pH for activity was 5.3, which characterized it as an acid phosphohydrolase. From a temperature standpoint, its optimum activity was 50 °C, thus indicating that it is fairly thermostable, which could be significant in certain food-processing conditions. Also, at 25 °C the enzyme was stable over a pH range of 3.5–7.8, which could also be advantageous in food processing.

As perhaps would be expected, the levels of phytase activity in the intestinal tract can also be an important factor in determining the degree of phytate breakdown (Nelson, 1967). For example, when Nelson et al. (1966c, 1971) added a microbial phytase extract to a chick diet, all the phytate was hydrolyzed in the alimentary tract.

Phytase activity relative to animal species has also been investigated. For example, McCollum and Hart (1908) reported phytase activity in the whole blood and liver of calves, whereas Rapoport et al. (1941) failed to find such activity in a number of mammals. Patwardhan (1937) first reported phytase activity in the rat, and Pileggi (1959) demonstrated that this activity was the highest in the intestinal mucosa of the rat. More recently, Bitar and Reinhold (1972) demonstrated phytase activity in the small intestine of the rat, chicken, calf, and man, but as pointed out by Rackis (1974), its role in phytate phosphorus metabolism is not well understood.

FOOD INTERACTIONS

Apparently, phytates are faily stable to heat (Anderson, 1914d). In the case of soy isolate, Rackis (1974) reported that autoclaving for 4 h at 115 °C is required to destroy most of the phytic acid, which from a nutritional standpoint would be unacceptable due to amino acid destruction. Under similar time/temperature conditions, only 20% of the phytate associated with sesame meal is destroyed (Lease, 1966), thus indicating that structural differences are apparent among products. de Boland et al. (1975) also found significant differences among products in phytate thermal destruction rates. Autoclaving for 30 min resulted in little phytate loss. However, after 2 h of autoclaving, approximately 70% of soy phytate was lost whereas rice, wheat, and sesame had only lost 5–25%. They also found that autoclaving soybean flakes for 2 h resulted in conversion of at least 10% of the inositol hexaphosphate to the pentaphosphate form, thus indicating that destruction of phytate was due to hydrolysis. Heating for 4 h resulted in the appearance of the tetraphosphate with the level of pentaphosphate being approximately equal to that of hexaphosphate. They also reported that 90% of the phytate in corn germ and soybean flakes was water soluble; however, the phytate associated with isolated soy protein was not water soluble, while only 10% of that associated with sesame meal was soluble. This unique property of sesame may be due to the fact that apparently sesame phytate is in the form of a magnesium phytate instead of the more common calcium phytate (O'Dell and de Boland, 1976) thought to be present in other products.

Because of nutritional considerations, extensive research has centered around phytate reduction and/or removal in soy products. Fontaine et al. (1946) pointed out that phytates should be considered an impurity in the isolation of protein and that when isolation of protein by means of isoelectric precipitation is used, a certain amount of phytate would also precipitate with the protein.

McKinney et al. (1949) evaluated several methods of removing phytate during soy processing. They evaluated acid leaching, which had been utilized earlier by Anderson (1920), but this resulted in protein degradation. In addition, the action of various solvents and dialysis systems were also investigated. From an experimental standpoint, they effectively reduced phytate levels, but all methods had little if any practical application.

Smith and Rackis (1957) removed phytate from soy by treating a water extract of soybean meal with a combination of dialysis and utilization of an anionic-exchange resin. They also reported that the removal of phytate from soy raised its isoelectric point upon acid precipitation and increased the pH range of produce dispersibility on the acid side of its isoelectric point. An influence of phytate on protein solubility has also been reported by Courtois and Barre (1953) and Bourdillon (1951), which in turn can apparently influence proteolytic digestion efficiency (Barre, 1956).

Saio et al. (1967, 1968) were concerned with the influence of phytate on soy protein functionality. They found that soy protein can easily combine with phytic acid in the presence of calcium even above its isoelectric point in a pH range of 5.7–6.0. However, these combinations were found to be labile, especially to heat, above pH 8.0. Wolf and Briggs (1959) also noted protein solubility decreases due to the interaction of calcium ions with protein and phytate.

Okubo et al. (1975) reported on a process to remove phytates from soy products, over a wide pH range, which required both dialysis and then ultrafiltration. At low pH calcium ions were found to induce phytate dissociation. At pH 5.0–5.5, incubation at 65 °C to induce indigenous phytase activity was utilized, and at pH 8.5, ethylenediaminetetraacetic acid was found to be effective. The binding properties of phytic acid to the major soy protein fraction, glycinin, as influenced by pH were further investigated by Okubo et al. (1976). At pH values of 6, 8, and 10, no binding was observed; however, between pH 5.0 and pH 2.5, binding resulting in insoluble complexes was apparent. The extend of binding was found to increase with decreasing pH. Ultrafiltration has also been recently suggested by Hartman (1979) and Omosaiye and Cheryan (1979).

de Rham and Jost (1979) concluded that from a practical standpoint the influence of phytate on soy protein solubility and its influence on the nutritional value of the protein itself are not measurable and thus can be neglected. They felt that differences in technological treatments in the actual isolation of soy protein had a more significant influence on the functional and nutritional properties. These conclusions were based upon their evaluation of the influences of pH, sodium chloride, calcium, and ethylenediaminetetraacetic acid on phytate and soy protein solubilities.

Treatment of a full fat soy flour slurry with calcium chloride and acid, followed by centrifugation, has also been suggested as a means to remove up to 90% of the phytic

acid with minimal effects on protein recovery or quality. However, the resulting product had a residual calcium concentration that was double that of the original product (Ford et al., 1978). Kaufman and Kleinberg (1971) have shown that phytic acid and its derivatives each have a different optimum pH for calcium binding, which can be used to explain why complete phytic acid removal was not achieved. In spite of all the above research relative to soy products, an efficient, practical method for the commercial removal of phytate is lacking.

The ability of phytic acid to complex with metals is well-known and is one of the main nutritional concerns associated with phytates. This aspect will be discussed in detail in a later section of this review. Its ability to complex with zinc is well established (Cheney et al., 1959; Maddaiah et al., 1964) with only copper apparently forming a stronger complex (Vohra et al., 1965). Similar results have recently been presented for fiber fractions that can be high in phytates (Thompson and Weber, 1979).

Phytic acid chemistry has also been implicated in the prevention of dental caries (Kaufman and Kleinberg, 1970) through its ability to bind with tooth calcium in the formation of resistant enamel.

NUTRITIONAL CONSIDERATIONS

Numerous studies have lead to the conclusion that phytic acid and its derivatives can bind essential dietary minerals, thus making them unavailable or only partially available for absorption. This problem becomes especially important when two or more cations are present in that a synergistic binding effect can occur. This seems to be particularily true for zinc and calcium and zinc and copper (O'Dell, 1969). However, as pointed out by Erdman (1979), in vitro studies implicating high-phytate foods with poor mineral utilization cannot be taken as 100% accurate since other components, especially fiber, can also play significant roles. Studies involving the incorporation of "pure" phytate are also open to question since chemical binding is not considered (Davies and Nightengale, 1975a,b) and phytic acid localization differs with product (Tombs, 1967). Perhaps at this point it would be appropriate to discuss the nutritional implications of phytate interactions with specific minerals. However, it should be noted that apparently all nutritionally significant minerals are influenced by some degree by phytate and thus it has been difficult to design meaningful studies to evaluate the effects of specific minerals independent of others.

Zinc. Erdman (1979) has reported that the greatest impact of phytic acid relative to human nutrition is its reduction of zinc bioavailability. This concept has been reviewed (Klevay and Hyg, 1975). This fact experimentally came to light in the mid 1950s in conjunction with soy protein (O'Dell and Savage; 1957; Morrison and Sarett, 1958; Moeller and Scott, 1958) and since that time extensive research has followed. Later studies (O'Dell and Savage, 1960; Forbes and Yohe, 1960; Forbes, 1964; Likuski and Forbes, 1964, 1965) concluded that an inverse relationship between the level of phytic acid in the diet and zinc bioavailability from the diet existed. O'Dell (1969) attempted to explain the variables that could influence zinc bioavailability, and later O'Dell et al. (1972a) measured zinc availability in various foods using both chick and rat assays. As expected, their data showed great variation among products and between species.

Most of the attention in this area has turned toward soy products since their use in food systems is expanding. However, similar problems have been observed with sesame and safflower (Lease and Wilhouse, 1967), mustard (McLaughlan et al., 1975), and rapeseed products (McLaughlan et al., 1975; Momcilovic and Shah, 1976a; Anderson et al., 1976; Shah et al., 1976, 1979). Lease (1967), in working with the chicks, reported that if soybean meal was fed, no added zinc was required for optimum growth. However, when fed two commercial soy isolates, 15–30 ppm of added zinc was required to optimize growth. It was postulated by O'Dell et al. (1972a) that the type of processing and not the actual amount of phytate caused this response. Thus, Rackis et al. (1975) have suggested the use of phytate-free soy to alleviate this potential problem. The use of a chelating agent for zinc, such as histidine, in soy isolate diets has been shown to minimize zinc-deficiency problems (Nielsen et al., 1966).

Polyphosphates other than phytate have been shown to also bind zinc (Vohra and Kratzer, 1966), and thus such food additives as hexametaphosphate, acid pyrophosphate, and tripolyphosphate can also cause potential zinc deficiencies.

Forbes and Parker (1977) demonstrated that zinc added to rat diets in the form of whole fat soy flour was significantly less utilized than zinc added as zinc carbonate to an egg white diet. In the case of soy-fortified wheat bread, Ranhotra et al. (1978) found that the bioavailability of zinc was not affected because most of the bound zinc was liberated due to the action of phytase on phytate during fermentation. Recently, Weingartner et al. (1979) found that the inclusion of soybean hulls, which are high in fiber, had not significant effect on the bioavailability of soy flour zinc although earlier studies by Ismail-Beigi et al. (1977) and Davies et al. (1977), utilizing bran as the fiber source, placed major emphasis on the role of fiber in binding zinc. Thus, apparently there are differences among product fibers.

The interaction of zinc, calcium, and phytic acid has also been investigated by using the pig (Oberleas et al., 1962) and rat (Oberleas et al., 1966) as models. High levels of calcium in conjunction with phytate were found to decrease zinc bioavailability. Similar interactions were found between zinc and copper (Klevay and Hyg, 1973; Davies and Nightingale, 1975a,b; Klevay, 1977).

The bioavailability of zinc to rats by using several infant formulas and breakfast cereals has been determined (Momcilovic and Shah, 1976b). Of all the products, the infant cereal was judged to be the poorest source of zinc. Reinhold et al. (1973a,b) have utilized human subjects in evaluations zinc bioavailability as influenced by phytate levels in the diet, and as expected, a positive correlation was found.

Calcium. Early reports indicated that cereals had anticalcifying and rachitogenic properties (Bruce and Callow, 1934; Harris and Bunker, 1935; Cruickshank et al., 1945; Walker, 1951). In the case of dogs, Mellanby (1949) demonstrated that phytate addition to their diets reduced calcium absorption and subsequently induced rickets. In contrast, Walker et al. (1948) reported that human diets high in phytates improved the retention of dietary calcium and magnesium. They felt that humans had the capacity to increase absorption efficiency. However, in a later study, Reinhold et al. (1973b) could not confirm human adaptation.

Forbes (1964) reported that in rats, dietary calcium depressed weight gain, feed intake, and femur zinc concentration, especially in the presence of soy protein. Likuski and Forbes (1965) showed that dietary calcium and phytic acid also decreased magnesium absorption. Dietary phytate has also been cited as a possible cause of magnesium deficiency (Roberts and Yudkin, 1960).

In the case of chicks, Nelson et al. (1968b) found that

when no phytate was present in the diet, a calcium requirement of 0.5% was required. However, when a natural diet containing 1.25% phytate was utilized, calcium requirements were increased to 0.95%.

The influence of phytic acid and its derivatives on inhibiting calcification in the rat was studied by Van Den Berg et al. (1972). They found that although phytate itself was inert, phytic acid and its hydrolysis products were potent inhibitors of calcification.

In the case of wheat breads, Reinhold et al. (1975) contended that fiber and not phytate is primarily responsible for poor calcium absorption. They reported that the ability to bind calcium is a function of fiber concentration.

Iron. Iron represents the other nutritionally significant mineral that has been associated with phytate binding, and although extensively investigated, numerous opposing data have appeared. For example, McCance and Widdowson (1943), Nakamura and Mitchell (1943), Sathe and Krishnamurthy (1953), and Davies and Nightingale (1975a,b) have shown phytate to be inhibitory to iron absorption, whereas others (Cowan et al., 1966; Callender and Warner, 1970; Ranhotra et al., 1974b; Welch and Van Campen, 1975) have shown no effect.

Morris and Ellis (1976) isolated monoferric phytate from wheat bran which they found to be water soluble and of high biological value to the rat. They also postulated that the monoferric phytate in bran was bound to cationic sites of proteins with utilization being through solubilization by an ion-exchange-type mechanism instead of through phytate hydrolysis.

Steinke and Hopkins (1978) reported that relative iron bioavailability from three commercially available soy isolates when fed to rats averaged 61%. In addition, autoclaving improved iron bioavailability in one of the three products. In general, iron absorption from soy products has been reported to be about 40–50% (Theuer et al., 1971, 1973; Layrisse et al., 1969) instead of the usual 10% or less normally associated with plant proteins. Likewise, Rotruck and Luhrsen (1979) reported iron bioavailability of 82–100% of ferrous sulfate from soy isolates, whereas iron from cooked beef had a bioavailability relative to ferrous sulfate of 26–55%. However, it should be noted that iron availability can vary dramatically among species.

Thus, in light of the above, general statements relative to phytate/mineral absorptions should not be made since apparently certain minerals behave differently. In addition, trace minerals such as copper, manganese, molybdenum, and cobalt are thought to affect oilseed phytates (Erdman, 1979).

METHODS OF ANALYSIS

The analysis of phytates can perhaps be considered somewhat primitive since to data no specific reagent or characteristic absorption spectrum has been found that would aid in quantitation. Basically, one has had to rely upon the measurement of inositol or phosphate as an indirect quantitation tool or upon the establishment of a stoichiometric relationship between phytate and some cation that is relatively easy to measure. Methodology utilized for the analysis of phytates up until 1971 was reviewed by Oberleas (1973).

Heuber and Stadler (1914) observed that phytate forms an insoluble complex with ferric ions in dilute acid, and this has been the basis for many analytical techniques. However, it has since been shown that other polyphosphates as well as some inorganic phosphate will also precipitate under these conditions, thus resulting in high values.

Currently, quantitative determination of phytic acid is based on the analysis of phosphorus or iron in the isolated ferric phytate (McCance and Widdowson, 1935; Crean and Haisman, 1963; Schormuller et al., 1956) or indirectly based on the determination of the residual iron in solution after the precipitation of ferric phytate from a known concentration of ferric salt in acid solution (Young, 1936). In any event, a wide variation in results can occur (Marrese et al., 1961; Anderson, 1963; Makower, 1970; Wheeler and Ferrel, 1971). Harland and Oberleas (1977) reported on a phytate quantitation procedure to be applied to textured vegetable proteins whereby an ion-exchange resin is utilized to remove inorganic phosphate contaminants. Paper chromatography has also been suggested as a means of separating and quantitating phosphate esters (Bandurski and Axelrod, 1951; Wade and Morgan, 1955).

Recently, two modified procedures have appeared that in theory should minimize some of the quantitation problems normally associated with phytate analysis. The procedure proposed by Uppstrom and Svensson (1980) involves the extraction of phytate with 15% Cl_3AcOH, which is followed by a 16-h room-temperature incubation involving enzymatic hydrolysis with phytase. The liberated phytate is then measured spectrophotometrically. Aside from the facts that a great deal of time is involved and that the procedure has not been evaluated on a wide variety of phytate-containing products, the method appears to hold a good deal of promise because of its relative simplicity.

However, a more promising method has been reported by Latta and Eskin (1980). This involves the extraction of phytate with 2.4% HCl for 1 h at room temperatue, followed by anion-exchange resin cleanup and reaction with ferric chloride/sulfosalicylic acid, the color reaction of which is measured spectrophotometrically. This procedure was compared to the traditional digestion procedure by using numerous phytate-containing sources and was found to give statistically similar results but is much more simple and rapid than tradiational digestion.

However, due to the fact that phytates can apparently exist in many bound as well as free forms, it becomes readily apparent that no one simple procedure can be utilized for effective quantitation.

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Egg and Egg Product Flavor

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The volatiles identified in eggs and egg products are discussed as well as the influence of storage, feed source, and extraneous odors on the flavor properties of eggs.

It has been reported that eggs from a confined bird on a standard commercial ration provides and egg of uniform and bland flavor (Swanson, 1977). On the basis of what is known concerning the basic composition of the egg (Powrie, 1977), one would assume that egg flavor should be fairly well defined at this point. However, surprisingly little is known about the actual composition of this flavor.

In addition, since eggs are reportedly bland, off-flavors can readily become detectable with the resulting organoleptic properties being described by using vague terminologies such as taint, stale, foreign, and flat. Extensive research has been performed in attempting to find means to minimize or eliminate these objectionable flavors, but this approach has been somewhat frustrating, probably due to the fact that the flavor chemistry of the egg and its fractions is far from understood.

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