The Effect of Seed Sprouting Time and Light on Phytic Acid Concentration

Sheryl J. Learned

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Abstract

THE EFFECT OF SEED SPROUTING TIME AND LIGHT ON PHYTIC ACID CONCENTRATION

by Sheryl J. Learned

Phytic acid was quantitatively analyzed in sprouted alfalfa, lentil, mung bean and soy bean seeds. Pulverized dry seeds served as a control and the various sprouting times were 12, 24, 48 and 72 hours after a 12 hour soaking. A comparison was made between sprouts grown in the light for the last 24 hours vs sprouts kept in darkened areas for the total growing time.

Analysis was based on dry weight and utilized the direct method in which 1) phytic acid was extracted by hot HCl, 2) ferric phytate was precipitated by adding an excess of ferric chloride to the extraction, 3) ferric phytate was converted to ferric hydroxide with NaOH, and 4) the amount of iron in the samples was determined colorimetrically using o-phenanthroline according to the method described in AOAC.

The results of the study showed a significant decrease of phytic acid by the end of the allotted sprouting time for all types of sprouts grown without light. The decrease in phytate was greatest the last 48 hours of sprouting time.

The present research agrees with previous research showing that phytic acid decreases as seeds sprout. It was concluded that the amount of phytate in seeds sprouted to the usual length used for human consumption is not likely to be detrimental to health.
THE EFFECT OF SEED SPROUTING TIME AND LIGHT ON PHYTIC ACID CONCENTRATION

by

Sheryl J. Learned

A Thesis in Partial Fulfillment
of the Requirements for the Degree
Master of Science in the Field of Nutrition

May 1977
Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

Kenneth I. Burke, Associate Professor of Nutrition and Dietetics

U. D. Register, Professor of Nutrition

James W. Blankenship, Professor of Nutrition

Grenith J. Zimmerman, Associate Professor of Biostatistics

Irma B. Vyhmeister, Professor of Nutrition
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INTRODUCTION

In countries where most of the dietary calories come from unleavened whole wheat bread, calcium and zinc deficiencies have been noted. This is thought to be due to the high phytate levels of the unleavened whole grain bread. Bread that has been leavened with yeast does not seem to cause these mineral deficiencies. Grains high in phytic acid bind calcium, iron, zinc and magnesium making these minerals unavailable for metabolism. Evidence indicates that human adaptation to high phytate intakes is minimal or does not actually take place at all. Since phytic acid is also found in appreciable amounts in seeds, it may be possible that calcium and zinc deficiencies may be noted in individuals who consume a substantial amount of their calories in seeds or sprouts.

Statement of the Problem

The purpose of this study was to determine whether or not the sprouting of seeds decreased their phytic acid content and therefore lower the possibility of mineral deficiencies. It was also the purpose to quantitatively determine the amount of phytic acid in sprouts used for human consumption and to determine the effect of time and light on the phytic acid content of the sprouts.
REVIEW OF THE LITERATURE

Phytic acid is the hexaphosphoric acid derivative of myo-inositol (Posternak, 1921), and is found in every kind of plant seed tested (Tanaka, et al, 1974, Asada, et al, 1968). To aid in the understanding of the subject a definition of key terms used in this paper are as follows: PHYTATE denotes a salt of phytic acid; PHYTIN is the trade name of the calcium-magnesium salt of phytic acid (Merck Index, 1968). However, the terms PHYTATE, PHYTIN and PHYTIC ACID seem to be used interchangeably in the literature. Proposed structures for phytic acid are shown in Figure I. The Neuberg structure (II) has 18 titratable hydrogens and was thought to be a likely structure. However, NMR studies on wheat phytate indicate that the Anderson structure (I) is most probable (Wheeler and Ferrel, 1971).

Historical Background

In 1855 and 1856 Hartig discovered that some small particles found in seeds were not starch grains. Later, in 1872, Brandon found these particles to contain calcium, magnesium and phosphorus but not nitrogen. Others, Palladin, Schulze, and Winterstein (1894-96), were able to precipitate a fairly pure compound rich in phosphorus and appreciable amounts of calcium and magnesium but no nitrogen, and in 1897 Winterstein suggested that the substance was inositol-phosphoric acid since hydrolysis yielded those two substances.

Posternak extensively studied this compound. He successfully
Figure 1. Proposed structures for phytic acid. NMR studies indicate that the Anderson structure (I) is most probable. (Wheeler and Ferrel, 1971).
purified it, and it was he who gave it the name of PHYTIN. However, he did not believe at first that inositol was present. Inositol's presence was confirmed in 1908 when phytin was mixed with phosphoric acid and distilled under reduced pressure (Rose, 1912).

During 1914 the study of interrelationships between phytates and dietary calcium began. Mellanby was involved in extensive research on the cause of rickets. He discovered that, in addition to a fat-soluble vitamin deficiency, cereals also could produce rickets especially if the diet contained too little of the newly discovered anti-rachitic vitamin. Oatmeal was particularly capable of producing rachitic symptoms whereas white bread was not (Widdowson, 1970).

Since that time to the present various studies have been undertaken to further understand the interrelationships of the various forms of phytate and their effect on various dietary minerals, namely calcium, zinc and iron. Recent studies have also aided the understanding of the role that phytate plays in plant metabolism.

**Calcium**

Dietary phytate and sodium phytate have been shown to bind calcium reducing its absorption in dogs (Hoff-Jorgensen, 1946) and also in humans (Bronner, et al, 1954, McCance and Widdowson, 1942a, b, Krebs and Mellanby, 1943, Hoff-Jorgensen, et al, 1946a, b). When chicks were studied similar results were obtained. If the phytic acid was hydrolyzed with phytase the calcium requirement was reduced, thereby confirming the chelating effect of phytic acid (Nelson, et al, 1968).

The experiments of Saio, et al (1968) demonstrated the rela-
tionships between soybean protein, calcium and phytic acid. In a dialysis system using CaCl₂, phytic acid and cold insoluble fraction protein obtained from soy beans, the phytic acid combined first with the calcium ion in solution and then with the protein. The higher the phytic acid content of the soy protein-CaCl₂ solution, the more calcium was bound to the protein. This indicates that phytic acid co-operatively reacts with calcium and protein and increases the binding of calcium to protein as the amount of phytic acid increases. This complex is insoluble and therefore calcium is not made available for metabolism.

In the chick, the amount of vitamin D in the diet also plays a role in the way phytate effects calcium and phosphorus metabolism. Phosphorus from calcium phytate, but not sodium phytate or phytic acid was available to a significantly greater extent when vitamin D was increased (Waldroup, et al, 1964b).

Others (Singsen, et al, 1950) suggest that under the influence of vitamin D₃ another radical replaces part of the phytate phosphorus, making the released phosphorus available for metabolism. Another theory is that vitamin D₃ increases the production of phytase or that phytase activity is increased either in the intestinal tract of the rats studied or in the feed ingredients to which vitamin D has been added. Pileggi, et al (1955) and Roberts and Yudkin (1961) support this theory, but offer no explanation of the mechanism of the increased activity mediated by vitamin D₃. Spitzer, et al (1948) does not agree with this theory.
Zinc

A number of workers (Moeller and Scott, 1958, Morrison and Sarrett, 1958, Smith, et al, 1962, Lease, et al, 1960, Forbes and Yohe, 1960) have established that zinc is more available in animal protein diets than in plant protein diets. It has been suggested by O'Dell and Savage (1960) that phytic acid must be in combination with protein in order to inhibit the availability of zinc. Others (McCall, et al, 1961) suggest that a structural feature of the protein causes the zinc to be bound. However, a study done on the availability of zinc when chicks were fed casein or amino acid diets resulted in the findings that when phytic acid was added the availability of zinc was decreased to the same extent for both the casein and amino acid diets (Likuski and Forbes, 1964). This study disagrees with the findings of O'Dell and Savage (1960).

Further study by Lease (1967, 1968) described a "carrier" in soybean oil seed meal which seems to be a better binding agent of zinc than phytate and is more stable. This "carrier" was able to form a complex with zinc that was water-soluble, dialyzable, and allowed zinc to be available for nutrition. Zinc-phytate complexes are not water-soluble and are therefore not available for nutrition. Isolated soy proteins did not contain this "carrier" and therefore chicks fed isolated soy protein diets without adding at least 15 ppm zinc suffered leg deformities.

In sesame meal there may be an isomer of phytic acid involved, and when freed from a protein bond allows zinc to be available. Another possibility is that there are several bonds of phytic acid holding the zinc and if one of these bonds is broken by autoclaving then the zinc
becomes available without disruption of the total phytic acid molecule. Still another possibility is that a substance other than phytic acid or protein is involved. Scott and Zeigler (1963) found natural chelates which aided chicks to be able to utilize zinc in the presence of phytic acid when the feed had been autoclaved. It is thought that the autoclaving process released the chelates. Lease (1966) found that autoclaving isolated soy protein for 4 hours resulted in the destruction of phytic acid and therefore zinc was free to be utilized. Welch, et al (1974) found that the availability of zinc to rats fed autoclaved mature pea seeds was not changed. Autoclaving mature pea seeds seemed to change the chemical form of phytic acid but the amount of phytic acid was not changed nor was the solubility of zinc altered.

If, through action of phytate, zinc is to be made unavailable, sufficient quantities of phytate must be present to compensate for the effects of dissociation and dilution in the stomach so that an insoluble compound will be formed in the intestine before absorption occurs (Oberlease, et al, 1966).

In the rat, dietary phytate decreases zinc availability. Excess calcium also decreases zinc availability if phytate is present. It seems that zinc, calcium and phytate form a three-way interaction, thereby forming the insoluble compound which causes zinc to be less available to animals (Oberleas, et al, 1966, Likuski and Forbes, 1965). If EDTA (ethylenediaminetetraacetate) is added, it appears to increase zinc availability by competing with phytate to form a chelated complex with zinc that is soluble and absorbable (Oberlease, et al, 1966).

It has been found that at low Zn:Ca ratios, absorption of zinc
into phytate was decreased, but at high ratios calcium did increase zinc absorption into phytate. This is explained by the fact that zinc is a trace mineral and does not supply sufficient cations by itself to form a precipitate of zinc phytate. But with an increase in calcium the calcium cations increase the binding capacity sufficiently to induce coprecipitation with zinc to form insoluble phytate complexes. Zinc deficiency is therefore increased as amounts of calcium are increased (Byrd and Matrone, 1965).

Blood is capable of forming a complex with zinc, but the complex is not as stable as that of phytate. This implies that the capacity of blood to bind zinc will significantly determine the amount of absorption of zinc from the intestine (Lease, 1968).

Iron

The interrelationship between dietary phytate and iron absorption is controversial. When immature and mature soybeans were fed to rats the iron absorption was greater for the mature beans even though the phytic acid content was also greater. The immature beans seemed to contain at least one factor that depressed iron availability. However, the rats used in this study were fasted and iron depleted. It is therefore possible that their condition was influential in the experimental results (Welch and Van Campen, 1975) because it is known that iron absorption is increased if iron stores are depleted (Van Campen, 1974).

Other studies in which rats were fed high phytate levels showed that iron absorption was not depressed (Cowan, et al, 1966). Various levels of phytate from wheat had little effect on iron absorption al-
though absorption did decrease with continued feeding (Ranhotra, et al, 1974). Human studies measuring iron absorbed from breads fortified with non-heme iron with or without bran resulted in a significantly lower iron absorption from the bread made with 3.3-10% bran as compared to white bread. Possible reasons given for these results were the increased inorganic phosphates and the increased phytate content due to the addition of bran (Björn-Rasmussen, 1974). The latter explanation is partially substantiated by the findings that phytates have been found to reduce non-heme iron, but not heme iron absorption (Hallberg and Solvell, 1967).

Other studies indicate that if the diet is low in calcium but high in phosphorus and phytic acid, less than 3% of the iron in the diet may be absorbed. If the diet is high in iron and/or calcium the limiting effect of the phytic acid and phosphate is reduced. High calcium and low phosphorus diets may decrease iron absorption, but if both calcium and phosphorus intakes are high iron absorption is increased (Nutrition Reviews, 1967).

Natural phytates allow more iron to be absorbed than sodium phytate (Hussian and Patwardhan, 1959).

**Magnesium**

Magnesium requirement studies of chicks (McWard, 1969) showed that when phytic acid was added to a purified glucose-isolated soybean protein basal diet the requirement for magnesium was increased to support optimal growth. This indicates that phytic acid binds magnesium and thereby reduces the availability of magnesium to the chick.
Dental Caries

Phytic acid has been implicated as a factor in dental caries prevention. It is thought that it adsorbs to enamel thereby reducing the enamel's solubility in acid. The low pH of plaque resulting from carbohydrate ingestion provides favorable conditions for phytate from the carbohydrate to be bound to the enamel (Jenkins, et al, 1959a, b). However, bread, either white or wholemeal, is found to already be of low cariogenicity as compared to sugar as judged by rat feeding studies. Adding bran to the bread diets did not significantly reduce rat caries (Grenby, 1966). Phytate found in the aleurone layer of bran was mainly responsible for the anticariogenic effect (Grenby, 1967). Topical application of sodium phytate prevented caries in the rat, although ingestion of sodium phytate in food or water did not decrease caries to a significant extent (Grenby, 1972).

Human Studies

Human studies to determine the extent of adaptation to high dietary levels of phytate showed that adaptation did not occur sufficiently to counteract negative effects. One study showed that even though subjects who have been raised on a high phytate diet, then change to a lower phytate intake for several years and then ingest a high phytate experimental diet of 2.5 g phytic acid per day, adapt to the experimental diet insignificantly and their calcium and zinc metabolism is adversely affected. Both calcium and zinc balances became negative (Reinhold, et al, 1973a). Another study showed that subjects acclimated to high phytate diets did retain high amounts of zinc, calcium and phos-
phorus when put on nutritious diets containing abundant quantities of these minerals in an available form. This study concluded that the subjects were severely mineral depleted due to the high phytate content of their usual diets. It has been estimated that the phytate intake of Iranian villagers range from 2 to 3.5 g per day (Reinhold, et al, 1973b). Other studies indicate that a certain amount of adaptation takes place, but it is limited (Hoff-Jorgensen, 1946). According to McCance and Widdowson (1949), utilization of calcium phytate or hydrolysis of phytic acid are two ways in which adaptation in humans may occur.

A study done in India showed that the chapatis, which are made of whole wheat, eaten by the people in northern India have a high phytate content and the incidence of nutritional osteomalacia is considerable. In southern India where rice is the staple cereal and socio-economic conditions are worse, nutritional osteomalacia is rare (Wills, et al, 1972).

Rickets and osteomalacia have also been reported among the Bedouin people of the Middle East. These people eat large amounts of Tanok (unleavened whole wheat bread) which is high in phytate (Berlyne, et al, 1973).

In Iran (Prasad, et al, 1960) and Egypt (Prasad, et al, 1963), it has been noted that there is a high incidence of zinc deficiency with resulting hypogonadism and arrested growth of adolescent youth. It was observed that all who were afflicted lived in villages where unleavened bread supplied most of the calories rather than in the cities where leavened bread was eaten.

Comparisons of the phytate content of the breads of the cities
and villages showed that the village breads did have a much higher phytate content than the city breads because the village breads were unleavened. The city breads were leavened and did not seem to precipitate mineral deficiency problems because yeast contains phytase which supplements the phytase naturally occurring in flour and hydrolyzes the phytic acid and releases the minerals needed for nutrition (Ranhotra, et al., 1974). The actual zinc intake of the population was well within recommended allowances. It was therefore concluded that the unleavened bread of the villages contributed to zinc deficiencies and also iron deficiency anemias and rickets which are frequently found in that region (Reinhold, 1971).

Other case studies of Indian immigrants to Britain report the occurrence of nutritional rickets due to ingestion of high levels of phytate derived from native bread (Wills and Fairney, 1972, Nutrition Reviews, 1973).

These case histories document the potential nutritional hazard of high amounts of phytate in the diet.

Availability of Minerals in Sprouted Seeds

The availability of minerals in seed sprouts has not been studied extensively. But from the studies that have been done it has been found that iron availability is greater after germination, and that in legumes there is a decrease in phytate phosphorus during germination (Singh and Banerjee, 1953, Belavady and Banerjee, 1953).

On a fresh weight basis the percent of total phosphorus as phytin was found to be as follows: wholemeal flour 46.8; white flour 14.7;
rolled oats 66.0; raw butter beans 46.3; and raw split peas 46.3. No phytin was found in green-leaf or stem vegetables or in the pulp of fruit (McCance and Widdowson, 1935). The percent of total phosphorus as phytic acid phosphorus based on dry weight was found to be 20 for lima beans, 37 for maize, 44 for millet, 59 for rice and 22 for soya (soybeans) (Oke, 1965).

Three possible roles for phytic acid in seeds have been suggested: 1) phosphorus storage (Hall and Hodges, 1966), 2) energy storage (Biswas and Biswas, 1965, Morton and Raison, 1963), and 3) drain of ATP. As the seed nears maturity phytic acid is rapidly synthesized which utilizes the ATP in the seed as a phosphorus supply causing seed metabolism to be inhibited and dormancy to ensue (Sobolev and Rodinova, 1966).

The role of phosphorus storage is undisputed. However, the other roles are still in question. Williams' (1970) work shows that in wheat endosperm there is no significant level of phytic acid. If phytic acid were to supply energy for germination a significant amount would be apparent in the endosperm. Other work has shown that respiration in wheat grains begins very early during germination before much phytic acid has been hydrolyzed (Negvogorova and Borisova, 1967). It was therefore concluded that stored energy in the form of phytate was not needed during this period of germination.

Phytic acid has also been proposed to be a phosphagen (reservoir of phosphate-bond energy) rather than a source of phosphorus at the time of germination (Asada, et al, 1969). Studies have shown that transphosphorylation between phytic acid and adenosine diphosphate occurs (Morton
and Raison, 1963) and an enzyme catalyzing transphosphorylation of phytic acid and guanosine diphosphate has been found in mung beans (Biswas and Biswas, 1965).

The theory regarding dormancy is also disputed by Williams. His results show that ATP falls to low levels approximately seven days after the maximum rate of phytic acid synthesis instead of falling markedly as phytic acid levels increase (Williams, 1970). However, work done on rice (Asada and Kasai, 1962) and potatoes (Samotus and Schwimmer, 1962) does suggest that the sharp rise in phytic acid before maturity does play a part in the onset of dormancy. It is thought that, rather than ATP removal, phytic acid affects cellular metabolism by chelating multivalent cations which are known to regulate cellular processes including the involvement of phosphotransferases which are utilized in energy metabolism (Bygrave, 1967).

Phosphorus is actively transported to seeds from leaves and roots during ripening, mostly in the form of phytic acid (Asada, et al, 1969). During seed formation glucose-6-P is converted to inositol-1-P and stepwise phosphorylated by phosphoinositol kinase to phytic acid (Majumder and Biswas, 1973). Suggested pathways for the formation of phytic acid are shown in Figures 2 and 3.

Inositol di, tri, tetra and pentaphosphates have been found not to be intermediates in phytic acid synthesis (Asada, et al, 1969, Loewus, 1969).

Makower (1969) found that phytic acid was accumulated mainly before the large increase in total solids of pinto bean seeds. During desiccation very little phytic acid was incorporated into the bean seed
Figure 2. Suggested pathway for the formation of phytic acid. (Loewus, 1969).
Inositol + phosphate donor $\rightarrow$ inositol monophosphate (inositol-P$_1$)

inositol-P$_1$ + X $\rightarrow$ X-inositol-P$_1$

X-inositol-P$_1$ + phosphate donor $\rightarrow$ X-inositol-P$_6$

X-inositol-P$_6$ $\rightarrow$ X + inositol-P$_6$ (phytic acid)

Figure 3. Suggested pathway for the formation of phytic acid. (Asada, et al, 1969).
solids.

Work done on *Phaseolus vulgaris* showed that when cotyledon formation occurred there was a rapid rise in phytate accumulation which slowed to a constant value during the last 10 days of embryogeny. There was also no net decline in phytin concentration during the first 4-5 days of germination (Walker, 1972).

Experimental work on oats show that during the first 5-6 days of germination phytate degradation is gradual. Thereafter the rate of degradation increases until the phytate phosphorus has disappeared completely at the end of two weeks (Ashton and Williams, 1958).

Crested wheat grass seeds studied in relationship to water potential, which is the ability of dry seeds to absorb water when placed to sprout on moistened fiber, showed that the concentration of phytic acid in dry seeds did not measurably change during 48 hours of soaking. But inositol-P esters, which in this research were studied to determine the phosphorylating reactions that take place during the first stages of germination, did increase, which suggested that some dephosphorylation of phytic acid was taking place (Wilson and Harris, 1966).

The role of phytase in the germinating seed is to release inorganic phosphate from phytic acid. Germinating seeds contain high amounts of phytase whereas other parts of vegetation or dormant seeds do not (Nagai and Funahashi, 1962, Peers, 1953, Sartirana and Bianchetti, 1967). The degradation of phytic acid by phytase is a stepwise process (Lim and Tate, 1973, Maiti, et al, 1974) and phytase is more prevalent in wheat, rye or barley than in oats (Widdowson, 1970).

Johnson and Tate (1969) show that there actually seems to be at
least two phytase enzymes; one cleaves the 1-phosphate; the other cleaves the 6-phosphate from phytic acid.

Work done on inorganic phosphorus and phytase shows that in wheat embryos phytase activity is inhibited by P$_i$. In the scutellum, puromycin or actinomycin completely inhibited an increase in phytase synthesis, and P$_i$ repressed its synthesis. In the germ, actinomycin delayed phytase synthesis, puromycin inhibited it and P$_i$ had no effect. Additional work shows that P$_i$ is effective in inhibiting phytase synthesis if it is supplied during phytase mRNA synthesis. It was therefore concluded that P$_i$ may inhibit phytase mRNA synthesis and that it works at the transcription level (Bianchetti and Sartirana, 1967, Sartirana and Bianchetti, 1967).
METHODS AND MATERIALS USED

The phytic acid content of seed and sprout tissue was determined via the following main steps: 1) maceration of tissue in hot HCl which solubilized and extracted the phytic acid at the same time; 2) precipitation of ferric phytate in the extract by adding an excess of ferric chloride; and 3) determination of phytic acid by conversion of ferric phytate to ferric hydroxide with alkali and the subsequent colorimetric determination of iron in the isolated ferric hydroxide.

Preparation of Sprout Samples for Analysis

Seeds of alfalfa, lentil, mung bean and soy bean were sprouted according to Method I in the New York Times Natural Foods Cookbook (Hewitt, 1971). All seeds were soaked for 12 hours in distilled water and then kept moist with distilled water for sprouting times of 12, 24, 48 and 72 hours. For the last 24 hours the sprouts were divided into two groups. One group was kept in the dark; the other was subjected to light. The sprouting containers were glass canning jars with nylon netting or cheese cloth, depending on the size of the seed, over the mouth of the jar to allow for ventilation.

At the end of the specified sprouting time, the sprouts were placed on dry ice for quick freezing, placed in plastic bags and kept frozen in a freezer until needed for analysis.

Analysis was based on 0.2 g dry weight. To accomplish this, seeds and sprouts were weighed before and after drying in an oven over-
night at 105° C. Calculations were done to determine amounts needed to equal 0.2 g dry weight.

Dry seeds were pulverized with mortar and pestil and used as a control.

**Extraction of Phytic Acid from Sprout Tissue**

The proper amount of pulverized seed powder or sprouts was placed in a small blender with 32 ml 0.5N HCl and ground until steaming (three to four minutes). The slurries were transferred to centrifuge tubes, four tubes for each kind of sprout. An additional 4 ml 0.5N HCl were added to rinse the blender container. The tubes were heated to 60° C in a hot water bath and shaken in a mechanical shaker for 40 minutes. The tubes were then centrifuged at 17,000 g for 30 minutes. The supernatants were decanted and put in 100 ml volumetric flasks. Another 5 ml of 0.5N HCl were added to the pellets in the tubes, shaken to mix well, heated, shaken and centrifuged again as described above. The supernatants were added to the volumetric flasks and refrigerated. The pellets were discarded.

**Precipitation of Ferric Phytate**

The acidity of the supernatants was adjusted to a pH of 2 with alkali, brought up to 100 ml volume with 0.5N HCl solution which had previously been brought to a pH of 2 with 10M NaOH, and mixed well.

Five ml of the solution were transferred to each of four centrifuge tubes for each kind of sprout. One ml of standard ferric chloride solution (about one mg Fe per ml in 0.375N HCl) was added per tube. An excess of iron in the reaction mixture was necessary for quantitative
precipitation of ferric phytate (Anderson, 1963, Early, 1944). Satisfactory precipitation was obtained with a mole ratio of added Fe to total P (Fe/P) of 4:3 or higher, and with a final acid concentration of 0.15N HCl. The tubes were mixed well and placed in boiling water for one hour, cooled, centrifuged at 17,000 g for 30 minutes and decanted.

The precipitate was washed twice with five ml 0.1N HCl, centrifuged as above and refrigerated.

Precipitation of Ferric Hydroxide from Ferric Phytate and Determination of Iron

The ferric phytate precipitate in the centrifuge tubes was dispersed in 0.5 ml water and was treated with 0.5 ml of 0.6N NaOH. After the contents had been mixed, the tubes were heated for 30 minutes in a boiling-water bath to coagulate the Fe(OH)₃ precipitate, cooled, centrifuged as above, and decanted again. The washed ferric hydroxide precipitate was dissolved in 0.5 ml of 0.5N HCl with heating in boiling water for 10 minutes, transferred to a 25-ml volumetric flask with several portions of 0.1N HCl, and made up to volume and to 0.1N HCl.

Ten ml of the mixture were transferred to a 25-ml volumetric flask. One ml of hydroxylamine was added, shaken to mix and let stand for a few minutes. Then 9.5 ml of 2M sodium acetate was added plus one ml of o-phenanthroline. The mixture was brought up to volume with distilled water and mixed well. Samples were analyzed for iron on a Gilford spectrophotometer at 510 nm (Horwitz, 1975, Association of Official Analytical Chemists, 1975).

Means and standard deviations were calculated. Linear regression lines were plotted by computer.
This method of analysis was adopted, adapted and utilized for determining small quantities of phytic acid in plant tissues. It is a faster and more convenient method than ashing, and interfering iron-containing material that coprecipitates with ferric phytate can be removed (Makower, 1970).

It was suggested by a member of the author's master's committee (Blankenship, 1976) that the step described by Makower involving the use of hot ethanol in macerating the seed tissues before extracting the phytic acid with HCl, be omitted. The method using hot ethanol was compared to the method omitting the hot ethanol. The omission saved much time and manipulation and no significant difference was noted in the end results between the two methods. (See Table X in the appendix.)
RESULTS AND DISCUSSION

The length of the sprouts was measured at the various time intervals. Alfalfa sprouts grew the most quickly and were ready for consumption by the end of the allotted sprouting time. Soy bean seeds did not sprout well and were not ready for consumption by the end of the 72 hours. Table I summarizes the growth patterns of the various seeds used in this experiment.

The Phytic Acid Content of the Sprouts

All sprouts decreased in phytic acid content by the end of the allotted sprouting time as shown in Figures 4-7. An exception is mung beans at 72 hours grown with light. Tables II and III show the phytic acid content of the various kinds of sprouts at the different time intervals. Significant decreases or increases of phytic acid were determined by t-tests in which values beyond the 5% level were considered significant. Table IV summarizes the t-test results for each sprouting interval and correlates with Figures 4-7. There was no significant decrease of phytic acid in alfalfa sprouts until 24-48 hours germination, and they were the only kind of sprout which did not show a significant increase in phytic acid at some time during the 72 hour germination period.

T-test results show that there was a significant decrease in phytic acid levels for all sprouts germinated without light for 72 hours.
TABLE I
The length of sprouts in millimeters vs sprouting time.
(Soaking time status is not included.)

<table>
<thead>
<tr>
<th>Hour</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72 without light</th>
<th>72 with light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>seed coat broken</td>
<td>2</td>
<td>12.5</td>
<td>25.5</td>
<td>23</td>
</tr>
<tr>
<td>Lentils</td>
<td>few with broken seed coat</td>
<td>broken seed coat</td>
<td>10</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Mung beans</td>
<td>half with broken seed coat</td>
<td>broken seed coat to 2.8</td>
<td>16</td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td>Soy beans</td>
<td>enlarged</td>
<td>broken seed coat</td>
<td>3</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

(Many beans did not sprout.)
Figure 4. Regression line for alfalfa sprouts.
Figure 5. Regression line for lentil sprouts.
Figure 6. Regression line for mung bean sprouts.
Figure 7. Regression line for soy bean sprouts.
### TABLE II

The phytic acid content of sprouts in millimoles per gram of sample vs sprouting time.

<table>
<thead>
<tr>
<th>Hour</th>
<th>0</th>
<th>12 (soak)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>0.01010</td>
<td>0.00949</td>
<td>0.00882</td>
<td>0.00916</td>
<td>0.00496</td>
<td>0.00378</td>
</tr>
<tr>
<td>Lentils</td>
<td>0.00826</td>
<td>0.01060</td>
<td>0.00739</td>
<td>0.00554</td>
<td>0.00686</td>
<td>0.00423</td>
</tr>
<tr>
<td>Mung beans</td>
<td>0.00697</td>
<td>0.00627</td>
<td>0.00896</td>
<td>0.00624</td>
<td>0.00770</td>
<td>0.00378</td>
</tr>
<tr>
<td>Soy beans</td>
<td>0.01780</td>
<td>0.01740</td>
<td>0.01780</td>
<td>0.01650</td>
<td>0.01540</td>
<td>0.01480</td>
</tr>
</tbody>
</table>

### TABLE III

The phytic acid content of sprouts in milligrams per gram of sample vs sprouting time.

<table>
<thead>
<tr>
<th>Hour</th>
<th>0</th>
<th>12 (soak)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>6.71</td>
<td>6.32</td>
<td>5.87</td>
<td>6.10</td>
<td>3.30</td>
<td>2.52</td>
</tr>
<tr>
<td>Lentils</td>
<td>5.50</td>
<td>7.05</td>
<td>4.92</td>
<td>3.69</td>
<td>4.57</td>
<td>2.82</td>
</tr>
<tr>
<td>Mung beans</td>
<td>4.64</td>
<td>4.18</td>
<td>5.95</td>
<td>4.16</td>
<td>5.13</td>
<td>2.52</td>
</tr>
<tr>
<td>Soy beans</td>
<td>11.80</td>
<td>11.60</td>
<td>11.90</td>
<td>11.00</td>
<td>10.30</td>
<td>9.85</td>
</tr>
</tbody>
</table>
### TABLE IV

Summary of the t-test results for each sprouting interval.

<table>
<thead>
<tr>
<th>Hour (soak)</th>
<th>0 to 12</th>
<th>12 (soak)</th>
<th>12 to 24</th>
<th>24 to 48</th>
<th>48 to 72</th>
<th>72 without light</th>
<th>Compared to 72 with light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>NS</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lentils</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mung beans</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Soy beans</td>
<td>NS</td>
<td>-</td>
<td>+</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### TABLE V

Summary of t-test results comparing 0 and 12 hours sprouting time with 72 hours with and without light.

<table>
<thead>
<tr>
<th>Hour to 72</th>
<th>0 to 72</th>
<th>12 to 72</th>
<th>12 to 72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lentils</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mung beans</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Soy beans</td>
<td>+</td>
<td>NS</td>
<td>+</td>
</tr>
</tbody>
</table>

+ corresponds to a significant decrease in phytic acid
- corresponds to a significant increase in phytic acid
NS corresponds to Not Significant at the 5% level.
sprouting time as compared to the dry seed controls. A comparison of sprouts germinated 12 hours and 72 hours without light also showed a significant decrease. However, in comparing dry seeds to sprouts germinated the last 24 hours with light there was a significant increase in phytic acid for mung beans and no significant difference was obtained for soy sprouts. These findings are summarized in Table V. The phytic acid content of the 72 hour sprouts grown with and without light is compared in Tables VI and VII. The raw data showing the means and standard deviations for the four samples of each kind of sprout is shown in Table VIII in the appendix. Final concentration values of iron are shown in Table IX. Figure 8 shows the standard iron curve.

A straight line relationship was assumed, but from this research it seems that the phytic acid is quite stable for the first 12 hours of sprouting time (24 hours total wet time) and then decreases. The phytic acid in mung beans seems to be quite stable until after 48 hours of sprouting time. Soy beans decreased in phytic acid the least. This is possibly due to their slow sprouting rate. Walker (1972) showed that Phaseolus vulgaris had no net decline in phytin concentration during the first 4-5 days of germination. Since this research was done on the basis of how a homemaker would sprout seeds, a longer time than 72 hours (84 hours total wet time) was not investigated.

Sprouts grown with light for the last 24 hours had a higher level of phytic acid than those sprouted without light, with the exception of alfalfa. It was also noted that all types of seeds used showed a marked greening when grown in the light for the final 24 hours compared to those kept in darkened areas. A possible theory explaining the difference in
**TABLE VI**

The phytic acid content of sprouts in millimoles per gram of sample comparing growth without light vs growth with light for the final 24 hours sprouting time.

<table>
<thead>
<tr>
<th>Hour</th>
<th>72 without light</th>
<th>72 with light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>0.00378</td>
<td>0.00059</td>
</tr>
<tr>
<td>Lentils</td>
<td>0.00423</td>
<td>0.00532</td>
</tr>
<tr>
<td>Mung beans</td>
<td>0.00378</td>
<td>0.00748</td>
</tr>
<tr>
<td>Soy beans</td>
<td>0.01480</td>
<td>0.01740</td>
</tr>
</tbody>
</table>

**TABLE VII**

The phytic acid content of sprouts in milligrams per gram of sample comparing growth without light vs growth with light for the final 24 hours sprouting time.

<table>
<thead>
<tr>
<th>Hour</th>
<th>72 without light</th>
<th>72 with light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>2.52</td>
<td>0.39</td>
</tr>
<tr>
<td>Lentils</td>
<td>2.82</td>
<td>3.54</td>
</tr>
<tr>
<td>Mung beans</td>
<td>2.52</td>
<td>4.98</td>
</tr>
<tr>
<td>Soy beans</td>
<td>9.85</td>
<td>11.60</td>
</tr>
</tbody>
</table>
Phytic acid content is that as the chloroplasts are developing, the metabolism of the sprouts is changed so that phytase is inhibited from breaking down phytic acid until the chloroplasts are fully developed and photosynthesis is established. When this takes place, growth recommences which then reactivates phytase and therefore the breaking down of phytic acid. Alfalfa sprouts decreased significantly in phytic acid when placed in the light. This decrease may be due to their fast growth rate.

Research data by Myer (1958), Chang (1967) and Matheson and Strother (1969) show a decrease in phytic acid content of lettuce, corn and wheat seeds respectively as germination progresses. Straight line relationships were not shown in these studies and this correlates with the present research. Lettuce seeds were depleted of phytic acid by 72 hours germination; corn still contained phytic acid at the end of 96 hours; and wheat seeds were not depleted of phytate until 14 days. It seems that very small seeds such as alfalfa or lettuce tend to be depleted of phytate much sooner than large seeds such as beans or grain. This may be due to the initial amount of phytic acid in each type of seed based on their individual weight or a higher metabolic rate.
CONCLUSIONS AND RECOMMENDATIONS

The findings of this research indicate that sprouts germinated to a length normally used for human consumption do not contain sufficient phytic acid to be detrimental to health. Other research indicates that one millimole of phytic acid binds 240 mg of calcium in order to be neutralized. The amount of calcium needed for the neutralization of phytic acid is in addition to the 800 mg which is the recommended daily dietary allowance of calcium (Butler, 1972). The amount of phytic acid in sprouts, which ranges from 0.00059 mM to 0.00748 mM per gram of dry sprout tissue (soy bean sprouts are not included in this range), is not enough to require more calcium for neutralization than that contained in a diet based on the Four Food Groups. Therefore, it appears that persons eating a mixed diet that contains good sources of calcium, iron and other essential nutrients for health would not likely be in danger of mineral depletion due to the amount of phytate ingested in sprouts.

Recommendations

Possible further studies include analyzing the phytic acid content of sprouts at closer time intervals such as every 4 hours instead of 12 or 24 hour intervals as done in this study.

Analyzing the length of sprouts vs phytic acid content would also be interesting. There may be a correlation between how fast sprouts grow and the decrease of phytate. A correlation of phytic acid destruction with sprout length divided by seed volume could also be done.
The effect of light on phytic acid destruction at various sprout lengths is another possibility. The data could be plotted so that insight into the type of enzymatic reaction could be gained.

Quantitative analysis of the phytic acid levels of purchased sprouts vs home grown sprouts is still another possibility.

A survey type study would involve assessing the actual amount of sprouts eaten by non-vegetarians, lacto-ovo-vegetarians and pure vegetarians. Interesting parameters would be the participants age, level of education and their life style.
SUMMARY

Phytic acid was quantitatively analyzed in sprouted alfalfa, lentil, mung bean and soy bean seeds. The method of sprouting used was based on how a homemaker would sprout seeds as described in the New York Times Natural Foods Cookbook.

Pulverized dry seeds served as a control and the various sprouting times were 12, 24, 48 and 72 hours after a 12 hour soaking, making the total wet time 84 hours. A comparison was made between sprouts grown in the light for the last 24 hours vs sprouts kept in darkened areas for the total growing time.

Analysis was based on dry weight and utilized the direct method in which 1) phytic acid was extracted by hot HCl, 2) ferric phytate was precipitated by adding an excess of ferric chloride to the extraction, 3) ferric phytate was converted to ferric hydroxide with NaOH, and 4) the amount of iron in the samples was determined colorimetrically using o-phenanthroline according to the method described in AOAC.

The results of the study showed a decrease of phytic acid by the end of the allotted sprouting time which ranged from 6.71 mg per gram of dry sample to 0.39 mg for alfalfa sprouts grown with light; 5.50 mg to 2.82 mg for lentils grown without light; 4.64-2.52 mg for mung beans grown without light; and 11.80-9.85 mg for soy beans grown without light. The decrease in phytate was greatest the last 48 hours sprouting time.

Sprouts grown in the light showed less of a decrease in phytic
acid than those kept in the dark with the exception of alfalfa sprouts. Phytic acid declined sharply in alfalfa sprouts grown in the light compared to the alfalfa sprouts grown without light.

The present research agrees with previous research showing that phytic acid decreases as seeds sprout. It was concluded that the amount of phytate in seeds sprouted to the usual length used for human consumption is not likely to be detrimental to health.
BIBLIOGRAPHY


TABLE VIII

The means of absorbance and the standard deviations for the four tubes per sample.

<table>
<thead>
<tr>
<th>Hour</th>
<th>0</th>
<th>12 (soak)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72 without light</th>
<th>72 with light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>297</td>
<td>280</td>
<td>262</td>
<td>270</td>
<td>152</td>
<td>119</td>
<td>28</td>
</tr>
<tr>
<td>St. Dev.:</td>
<td>16.87</td>
<td>14.76</td>
<td>8.19</td>
<td>4.65</td>
<td>47.80</td>
<td>7.70</td>
<td>4.73</td>
</tr>
<tr>
<td>Lentils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>246</td>
<td>310</td>
<td>220</td>
<td>168</td>
<td>204</td>
<td>131</td>
<td>162</td>
</tr>
<tr>
<td>St. Dev.:</td>
<td>9.49</td>
<td>12.15</td>
<td>2.06</td>
<td>4.65</td>
<td>4.18</td>
<td>17.44</td>
<td>9.54</td>
</tr>
<tr>
<td>Mung beans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>209</td>
<td>188</td>
<td>258</td>
<td>186</td>
<td>229</td>
<td>119</td>
<td>223</td>
</tr>
<tr>
<td>St. Dev.:</td>
<td>4.73</td>
<td>6.90</td>
<td>13.03</td>
<td>10.50</td>
<td>10.58</td>
<td>11.87</td>
<td>4.11</td>
</tr>
<tr>
<td>Soy beans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>515</td>
<td>504</td>
<td>516</td>
<td>478</td>
<td>448</td>
<td>429</td>
<td>503</td>
</tr>
<tr>
<td>St. Dev.:</td>
<td>12.03</td>
<td>6.70</td>
<td>8.30</td>
<td>16.67</td>
<td>30.46</td>
<td>20.66</td>
<td>11.32</td>
</tr>
</tbody>
</table>
TABLE IX

Determination of phytic acid by quantitative iron precipitation.

$\mu g/mg \text{ Fe}/0.0016 \text{ dry weight sample}$

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>12 (soak)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72 without light</th>
<th>72 with light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>3.60</td>
<td>3.39</td>
<td>3.15</td>
<td>3.27</td>
<td>1.77</td>
<td>1.35</td>
<td>0.21</td>
</tr>
<tr>
<td>Lentils</td>
<td>2.95</td>
<td>3.78</td>
<td>2.64</td>
<td>1.98</td>
<td>2.45</td>
<td>1.51</td>
<td>1.90</td>
</tr>
<tr>
<td>Mung beans</td>
<td>2.49</td>
<td>2.24</td>
<td>3.20</td>
<td>2.23</td>
<td>2.75</td>
<td>1.35</td>
<td>2.67</td>
</tr>
<tr>
<td>Soy beans</td>
<td>6.35</td>
<td>6.22</td>
<td>6.37</td>
<td>5.89</td>
<td>5.52</td>
<td>5.28</td>
<td>6.21</td>
</tr>
</tbody>
</table>

Formula for calculations:

\[
\frac{\mu g \text{ Fe}}{1000 \mu g \text{ Fe/mg Fe}} \times \frac{1}{\text{mg Fe/mM Fe}} \times \frac{1}{4 \text{mM Fe/mM phytic acid}} \times \frac{666 \text{mg phytic acid/mM phytic acid}}{0.0016 \text{g dry tissue}} = \text{mg phytic acid/g tissue}
\]
TABLE X

Comparison of methods with and without ethanol for the extraction of phytic acid. (Mung bean sprouts were tested.)

<table>
<thead>
<tr>
<th></th>
<th>Means of the spectrophotometer readings</th>
</tr>
</thead>
<tbody>
<tr>
<td>With ethanol</td>
<td>203</td>
</tr>
<tr>
<td>Without ethanol</td>
<td>214</td>
</tr>
</tbody>
</table>

These figures are not significant at the 5% level.
TABLE XI

Per cent moisture of sprouts grown 72 hours without light.

<table>
<thead>
<tr>
<th>Kind</th>
<th>Moisture Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>92.0%</td>
</tr>
<tr>
<td>Lentils</td>
<td>73.5%</td>
</tr>
<tr>
<td>Mung beans</td>
<td>80.0%</td>
</tr>
<tr>
<td>Soy beans</td>
<td>68.5%</td>
</tr>
</tbody>
</table>

TABLE XII

Weight in grams of fresh commercially grown sprouts measured in household measuring cups.

<table>
<thead>
<tr>
<th>Kind</th>
<th>Amount</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>1/2 C</td>
<td>17</td>
</tr>
<tr>
<td>Mung bean</td>
<td>1 C</td>
<td>52</td>
</tr>
</tbody>
</table>