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EFFECT OF PHYTIC ACID IN WHOLE WHEAT BREAD
ON IRON ABSORPTION

by

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INTRODUCTION

There is a high incidence of iron deficiency anemia in the United States (1) and throughout the world (2). The United States cereal enrichment program has been effective in reducing vitamin B deficiency, but has had little effect on reducing the incidence of iron deficiency (3). The consumption of whole wheat products is increasing in the United States (4), but it has been recognized for many years that the absorption of polyvalent cations including iron is lower from whole wheat than from white bread. This lower absorption of iron has been attributed to the existence of phytic acid in whole grain products.

Phytic acid is a compound formed by the union of one molecule of inositol with six molecules of phosphoric acid. It is present in large amounts in the bran and germ portions of cereal grains. Phytic acid, by virtue of its ability to form insoluble and unabsorbable iron salts in the intestinal tract may interfere adversely with iron absorption. The effect of phytic acid on iron absorption may be a serious problem in areas of the world where the people consume large amounts of unrefined cereals and small amounts of iron.

Widdowson and McCance (5) observed a lower retention of iron in human subjects consuming bread made of 92% extraction wheat than in subjects on white bread. They suggested that this effect might be due to the high phytic acid content of the brown bread. Later, by using the elevation of serum iron as an indication of the amount of iron absorbed, they found when sodium phytate was added to bread the serum iron of subjects was significantly below the normal level (6).

The enzyme, phytase, has the power to hydrolyze phytic acid into inositol

and phosphoric acid. Therefore, the adverse effect of high extraction flour products upon iron absorption may be reversed by phytase action during preparation of foods. Since yeast is an excellent source of phytase, it might be possible to prevent the precipitation of insoluble iron-phytate salts by destroying phytic acid during the process of yeast fermentation of bread.

The present study was undertaken to determine whether the phytase in yeast leavened whole wheat bread does destroy phytic acid during the fermentation process and whether such destruction may consequently change the bioavailability of iron in the bread.

REVIEW OF LITERATURE

IRON

Absorption and metabolism of iron. Food iron is absorbed in two forms, heme and nonheme iron (7,8). Heme iron is derived from hemoglobin as it is absorbed into the mucosal cells. Within the cell the porphyrin ring is split open and iron released to pass into the blood stream. Heme iron is highly available and is not affected by the composition of the diet. Nonheme iron is derived from elemental iron or iron salts in food. Nonheme iron is far less available and its absorption is greatly influenced by other substances in the food. Absorption of nonheme iron is best in the presence of a food with heme iron (e.g. beef) and poorest in the presence of a food with nonheme iron (e.g. maize) (9).

Iron absorption is greater when it is available in the ferrous form than in the ferric form. The ferric form that predominates in food is usually reduced to the ferrous form prior to absorption (10). Uptake of iron by the intestinal mucosal cell is unidirectional; there is practically no excretion of iron into the gut (11). Urine contains very small amounts of iron except in cases of pathological leakage of blood into the urinary tract. Fecal iron is mostly the unabsorbed iron taken in by mouth. Once iron is absorbed into the body, it is carefully conserved by recycling. Thus, the main control of iron balance involves the cells of the intestinal mucosa which are known to respond to changes in iron stores or intensity of red cell production by absorbing more or less of the iron available in the digestive tract (12). When ferrous ions enter the mucosal cell, apoferritin, an acceptor protein is produced. After entry into the cell, the ferrous ions are oxidized to the ferric state by ferroxidase at the luminal border, and the ferric ion combines with apoferritin to form ferritin (13). At the vascular border of the cell

the ferritin is reduced by a different redox environment with consequent release of ferrous ions which emerge from the cell into the blood stream, where it is reoxidized to ferric form. The ferric ions then combine with transferrin, which transports it to various tissues (14). Thus, the need for iron for hemoglobin synthesis has led to the development of an efficient iron mobilization (ferroxidase) and transport (transferrin) system.

Current measurements of iron availability. Total iron in the body of a normal adult is between three and four grams, distributed approximately as follows: hemoglobin of the red blood cells, 65 to 70%; ferritin and hemosiderin (the storage form of iron), about 25% and myoglobin of the muscles, 3 to 5%. The small remainder is contained in the transferrin of the blood plasma and in several iron-containing enzymes which are active in cellular oxidations. Although all of these iron-containing substances exchange their iron, the myoglobin and cellular enzymes do not reflect as large a fluctuation as hemoglobin, transferrin and body iron stores. In iron deficiency, the body iron stores are first depleted. This also is reflected in the extent to which transferrin is saturated. Ultimately, lack of available iron affects the hemoglobin level in the blood. When sufficient iron is provided in the diet or administered orally, hemoglobin will regenerate until it has reached a normal range. Once the hemoglobin is restored, iron will be available for body iron stores and transferrin.

The hemoglobin regeneration method is used to measure bioavailability of dietary iron because of its sensitive response to iron concentration (16). Chicks or rats are made anemic on a low-iron basal diet. The anemic chicks or rat then are fed graded levels of ferrous sulfate as a reference standard and comparable quantities of iron from the test sources. After two or three

weeks on the test diets, hemoglobin and hematocrit are measured as criteria of absorption response to the dietary iron.

Factors affecting iron absorption from cereal products. The problem of iron deficiency in the United States has been recognized for many years (1,3,17). Food consumption surveys (18) indicated that the present day diet in the United States provides not more than an average of 6 mg of iron per 1,000 Kcal, an amount that fails to meet the level of the recommended dietary allowance for some groups, especially infants and women during pregnancy and lactation (19). Besides the low dietary intake of iron, there are several other factors which affect iron utilization. Internal factors include body iron stores, saturation of transferrin, degree of erythropoiesis, frequent infections and infestations, and chronic occult blood loss (20). External factors include amounts of natural iron in foods and availability of iron that is present in foods.

Poor absorption of iron from bread has been ascribed to a variety of factors including binding of iron by phytate (21,22), inhibition of absorption by calcium and phosphorus (23), or chelation of the iron with other substances in the grain which makes iron insoluble and unavailable for absorption. On the other hand, good absorption of iron from meats has been related to the complexing of the mineral to the porphyrin structure of hemoglobin and myoglobin (24).

Ingestion of other foods along with bread alters the rate of absorption of iron from bread itself. Thus, the enhancement of iron absorption from bread consumed with orange juice is ascribed to the ascorbic acid in the juice (25,26). The reduction in iron utilization when egg is included in the meal with bread is attributed to the iron-binding protein, phosvitin, in egg (27). Layrisse et al. (28) found that addition of sulfur-containing amino acids to

black beans markedly increased absorption of iron from the legume. Similarly, Miller (29,30,31) found that addition of methionine to bread increased iron deposition in the spleen and sometimes increased hematocrit and serum iron concentration.

Iron salts added to milled wheat flours to restore them to the same content occurring in whole wheat products may be poorly absorbed (32). Pla and Fritz (33) compared the availability of 21 iron compounds used to fortify cereal grain products with that of ferrous sulfate by evaluating the regeneration of hemoglobin in chicks and rats depleted of iron. They found that the dihydrogen ferrous salt of EDTA, ferric ammonium citrate, ferric choline citrate, ferric glycerophosphate, ferric sulfate, ferric citrate, ferrous ammonium sulfate, and ferrous tartrate were from 75% to 100% as available as ferrous sulfate for hemoglobin regeneration in depleted chicks and rats. In contrast, such compounds as sodium iron pyrophosphate, ferrous carbonate, ferric oxide, and ferric orthophosphate were poor sources of iron. Ferric pyrophosphate and reduced iron were graded as mediocre sources of iron. A similar study (34) on the hematinic effect of various iron sources also showed that the mean hemoglobin concentration at the end of a repletion period was significantly greater in rats receiving a diet with ferrous sulfate than in rats receiving a diet with reduced iron or sodium iron pyrophosphate.

Elwood et al. (25) made bread by a commercial method; they added ferric chloride to some samples and ferric ammonium citrate to others. They found iron naturally present in white bread was as well absorbed by healthy persons as that from salts. With anemic individuals, iron from salts was better absorbed. They also found that when iron was added to whole wheat and white bread (71% extraction flour), 18% of the total iron in white bread was

absorbed as compared to 7% of that in the whole wheat bread.

PHYTIC ACID

Properties of phytic acid. There has been much discussion concerning the role of fiber in the diet to prevent atherosclerosis, diverticulosis, and colonic cancer and to reduce blood cholesterol level (35). However, when fiber is increased in the diet it is usually accompanied by an increase in phytic acid (36).

Phytic acid, myo-inositol phosphate, is the highest analog of a series of inositol phosphates found widespread in nature (37). It occurs in fairly high levels in grains, oilseeds (38), rapeseeds, legumes, nuts, vegetables and tubers as calcium and magnesium salts, known as phytin. These salts, as well as the phosphates, are insoluble and quite unavailable in the intestine of many animals (39). Bailey (40) stated that phytic acid is a phospho-organic acid that contains 70-90% of the phosphorus in grains. The free acid is most soluble in water, less soluble in alcohol, and insoluble in ether, benzene, chloroform and glacial acetic acid. Inositol and phosphoric acid were successfully recovered from this phospho-organic acid. He concluded that the salts of the acids seemed to be of magnesium, calcium, a little iron and manganese.

The structure of phytic acid was proposed by Anderson in 1912 (41). This structure was challenged at first, but with the utilization of nuclear magnetic resonance (42) it was shown to be the most probable structure which would favor the binding of ferric ions. The new nomenclature for phytic acid is myo-inositol 1,2,3,4,5,6-hexaphosphate (37).

Paper chromatography has proved to be the most popular method for qualitative separation and identification of inositol phosphate ester. Electropho-

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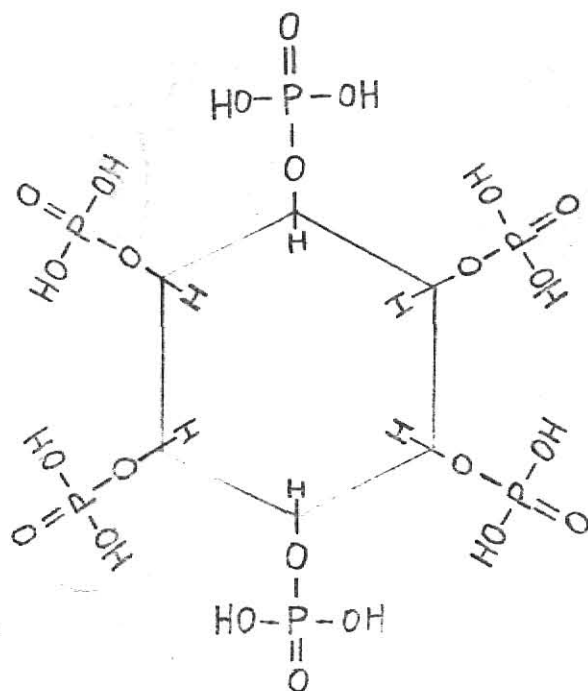


Fig. 1 Structure of phytic acid

resis, ion exchange chromatography, and thin layer chromatography also have been used. (37).

Quantitative determination of phytic acid involves the following steps:

- 1) solubilization and extraction of phytic acid with a strong acid; 2) precipitation of ferric phytate in the extract by adding an excess of ferric chloride; and 3) determination of phytic acid by one or more of four procedures (a) measuring the amount of ferric ion remaining in solution after the precipitation of ferric phytate (43); (b) conversion of the ferric phytate to ferric hydroxide with alkali and the subsequent colorimetric determination of iron in the separate hydroxide (44,45); (c) wet-ashing of the precipitated ferric phytate and measuring the amount of iron (46,47); or (d) the amount of phos-

phorus present (37).

Most recent methods are modifications of the technique of McCance and Widdowson (46), who rejected the earlier titrametric procedures because of unsatisfactory end point determination and analyzed for phosphorus in the ferric phytate precipitate. They extracted phytate with HCl whereas other workers have used a trichloroacetic acid (TCA) solution. Wheeler (44) compared the effectiveness of these two extractants on a series of commercially prepared wheat protein concentrates from various sources. They found more phytate phosphorus in TCA extracts. In addition, the TCA extracts were much clearer than those with HCl. When HCl extracts were neutralized, a large amount of floccular white precipitate formed, whereas in TCA extracts only traces were present. Extracting 30 minutes with mechanical shaking or 45 minutes with occasional hand swirling was as effective as longer extraction times. Three percent TCA was as good as the five percent TCA used by DeLange et al. (48).

Direct determination of phosphorus in the $\text{Fe}_4\text{phytate}$ precipitate requires a time consuming digestion. The procedure can be simplified by determining iron colorimetrically and calculating phytate phosphorus from this value while assuming a constant ratio of 6 atoms P to 4 atoms Fe in the precipitate (44,47).

The direct determination of iron in the precipitate by measuring residual iron in solution was unsatisfactory for analyzing small amounts of phytic acid present in plants (45). Hydrolysis of inositol from the phosphate and determination of inositol has been another method (37).

Phytic acid in the diet. The salts of phytic acid in wheat serve several useful purposes. Wheat phytates have a laxative action on the bowels (49). In addition, wheat contains 1 ppm of lead so that the person consuming large quantities of wholemeal bread or bran might in time suffer from mild lead

poisoning were it not for the fact that lead is almost certainly present as insoluble lead phytate (50).

On hydrolysis, phytic acid also supplies the body with phosphorus and inositols. Common (51) reported that phytic acid phosphorus accounted for two thirds to three fourths of the total phosphorus in cereal grains. Nelson (52) in his review of phytate phosphorus utilization by poultry stated that under certain conditions, phytase enzyme which is present in certain feed ingredients and possibly secreted by the intestine will hydrolyze dietary phytate phosphorus and make it available to animals.

Phytic acid helps the body avoid some toxic materials and reserves some of the minerals from loss by complexing with them. In contrast, phytic acid will also harm the body by making some minerals unavailable for intestinal absorption. These minerals include calcium (51,53,54,55,56,57,58); magnesium (39,59); zinc (60,61,62,63) and iron. Therefore, the presence of phytic acid in the diet has advantages as well as disadvantages.

Phytic acid and iron absorption. Cereals contain an appreciable amount of iron. For example, white flour contains about 1.4 mg per 100 grams and 92% extraction flour about 3.5 mg per 100 grams (50). This iron has generally been regarded as available, and therefore, brown bread was often advised as a valuable source of iron (64). But it was found that people eating brown bread absorbed less iron than those eating white bread (50,62). This was related to the presence of phytic acid in the bran of the whole wheat (50,25). Phytic acid was reported to interfere with iron absorption in monogastric animals as a result of its ability to complex with multivalent cations such as iron.

In human studies Widdowson and McCance (5) showed that iron was absorbed much less readily from brown than from white bread. McCance et al. (6) also

observed that the effects of brown bread on mineral metabolism could be reproduced by adding sodium phytate to white bread and measuring its effect on the absorption of various amounts of ferric and ferrous ammonium sulfate. Iron absorption was measured by determining the increase in serum iron produced during the 8 hours after ingestion of the iron salt being tested. With this method an increase in the serum iron value established the fact that iron was absorbed (the rise in serum iron was proportional to the degree of iron absorption), but it did not measure the exact amount of iron assimilated. Comparison was made of the effect of sodium phytate and disodium hydrophosphate (Na_2HPO_4) on iron absorption. When sodium phytate was fed along with iron, the rise in serum iron was prevented. This occurred both with ferrous and ferric ions, but the effect was more constant and greater in the latter. While there was a definite interference of iron absorption in the presence of sodium phytate, there was still a considerable absorption of iron. This experiment indicated that sodium phytate added to bread decreased iron absorption. In the opinion of McCance et al. (6) the phytate in whole grain cereals should have a similar effect and would be capable of combining with iron present in the rest of the diet, and thus inhibit its absorption.

McCance and Widdowson (65) found that hydrolysis of the phytates in whole wheat bread to phosphates led to improved absorption of Ca, P, and Fe, but the absorption was still not as good as it was from white bread diets. If most of the phosphorus also was removed and a dephytinized and demineralized loaf was prepared, iron absorption was almost the same as from white bread even though the laxative effect was very different. They concluded that the poor absorption of iron from brown bread must be attributed to the specific action of phytates. They observed that phosphorus had a similar but smaller inhibitory effect.

These results were consistent with those of Nakamura and Mitchell (66) who measured the utilization of ferric phytate in anemic rats. They found that anemic rats utilized the iron of ferric phytate only about one-half as well as the iron from ferric chloride.

Ferric phytate is insoluble even in 6 N HCl and has been shown (67) to be relatively unavailable as a source of iron even when taken in large amounts. Twelve grams of ferric phytate (containing 1 g iron) had less therapeutic effect in the treatment of iron deficiency anemia in human subjects than did 0.8 g of ferrous sulfate per day (containing less than 0.3 g iron).

In contrast to the effect noted in humans, added sodium phytate has been reported by Fuhr and Steenbock (68) to have no or a slightly depressing effect on utilization of iron by rats. However, Davis and Nightingale (69) reported that sodium phytate added to the diet of rats significantly reduced whole body retention of iron.

Human balance studies were performed by Hussian and Patwardhan (70) to see the effect of phytate on the absorption of iron in a mixed vegetarian rice and wheat diet with varying phytate content. When phytate phosphorus was increased from 8% to 40% of the total dietary phosphorus, iron absorption was reduced from 11% to 3%.

Sathe and Krishnamurthy (71) conducted experiments with young albino anemic rats to find out whether there is any effect of phytic phosphorus in the diet on the absorption of iron by the body. Unpolished, 7% polished, and 15% polished rice were used as the source of different amounts of phytic phosphorus. Iron was supplied in equal amounts as ferric citrate in a salt mixture. Hemoglobin level, weight of the rats and the storage of iron were studied. They found that the increase in hemoglobin was maximum in all groups

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during the first week and became less and less during subsequent weeks. Hemoglobin increased more as the phytic acid content of the diet decreased. Also, the amount of stored iron increased as the content of phytic acid of the diet decreased. The authors concluded that phytate phosphorus inhibits the absorption of dietary iron by nutritionally anemic rats. However, in their experiments they did not present either the phytate or the iron content of the diets used, and the differences in values for hemoglobin regeneration among their groups of rats were not statistically significant. In vitro, Vohra et al. (72) proved that on titrating sodium phytate against metal ions at pH 4.7, complexes were formed in the following decreasing order of activity : Cu, Zn, Ni, Co, Mn, ferric ion and Ca.

In a survey of health and nutrition of a rural population of southern Iran, Haghshenass et al. (73) reported the amount of iron in the village dietary averaged 44.4 mg/day, far in excess of the 18 mg recommended in the revised American dietary allowance. However, iron deficiency anemia was prevalent in the Iranian village population. The bread eaten by the villagers was made from flour of high extraction and without added leavening yeast. The large amount of unleavened wholemeal bread consumed by the villagers led to phytate intakes of more than 2 g everyday. Most of the iron of the village diet existed as complexes with phytate. Therefore, the poor iron availability brought about by high intakes of phytate-rich unleavened wholemeal bread appears to have been a major cause in the etiology of iron deficiency anemia among Iranian villagers. The addition of moderate amounts of iron to the diet appeared to overcome the action of the phytate.

All of the foregoing studies have indicated that phytic acid interferes with iron absorption. However, further examination of the literature raises

the question whether the endogenous or native phytate of foods such as wheat, does in fact, have a deleterious effect on absorption of native food iron. Callender and Warner (64) compared brown and white bread, both containing only the endogenous phytate, and found no difference in the absorption of iron from the two kinds of bread. Cook et al. (74) found the absorption ratio of biosynthetically tagged native wheat iron to tagged supplemental iron baked into the same rolls to be 1.2; that is, the native wheat iron was absorbed to a greater extent than the added iron.

Sharpe et al. (75) found that sodium phytate added to the diet decreased absorption of ferric chloride fifteen-fold, but there was no correlation between the phytate content of foods and the reduction of iron absorption in their studies with radioactive iron. Milk, which contains no phytate, reduced absorption of ferric chloride as much as rolled oats which contains significant quantities of phytate. Rolled oats with milk reduced iron absorption only twice as much as milk alone and a 40% reduction of rolled oats intake did not materially favor iron absorption. A progressive decrease in iron absorption occurred with each increase in quantity of the different foods ingested. It therefore seemed that natural phytate is not an important factor in reducing iron absorption. Some other factors such as increasing the bulk of dietary intake must have interfered with iron absorption.

Cullumbine et al. (76) compared iron retention of diets of polished and unpolished rice and found no consistent effect of varying phytic phosphorus intakes on iron balance. Foy and Kondi (77) found that the effect of phytic acid on human iron absorption in cases of severe iron deficiency was variable. In some cases the expected hemoglobin and red blood cell response occurred on a daily dose of 30 mg ferrous sulfate, despite the administration of 1000 mg

phytic acid. Whereas in other cases, no response occurred until the phytic acid was stopped.

Apte and Venkatachalam (78) found an increase in calcium intake counteracted the deleterious effects of excess dietary phosphate and phytate and made more iron available for absorption. They studied iron absorption at varying levels of calcium intake, maintaining a constant phytic phosphorus intake of 400 mg, which is more than is contained in the ordinary Indian diet, 16.6 mg of dietary iron intake per day was not enough to maintain a positive iron balance. But a delicate iron balance was achieved on the same iron intake with an increase in calcium intake to 1000mg daily. If the intake of calcium was increased to 1500 mg with a daily iron intake of 15.6 mg, iron retention was 7% to 28%. The results revealed that iron absorption was more dependent on calcium intake than on phytate intake. An adequate intake of calcium seems to play a more important role in iron absorption than the content of dietary phytate. It is possible that calcium, iron, phosphates, and phytates are mutually interacting. Changes in one affects the absorption of the others and no single one can be regarded as exclusively concerned in hindering or promoting iron absorption.

To examine the effect of naturally occurring and added dietary phytic acid on the availability of iron and phosphorus and its relationship to intestinal phytase activity, Ranhotra et al. (22) fed bread-based diets to rats for 6 weeks. Increasing the level of dietary phytate by increasing the sodium-phytate content did not appear to interfere substantially with the availability of iron naturally occurring in wheat when the other ingredients, protein, Ca, Fe, P, and vitamin D were identical. Intestinal phytase activity was not induced by dietary phytate. The authors suggested that the availability of

iron may not be affected by a somewhat increased consumption of natural phytate in the form of cereals if the diet is low in bulk and adequate in Ca, vitamin D and iron. This experiment agreed with the findings of Cowan et al. (21) who examined the effect of sodium phytate on iron absorption by measuring total hemoglobin regeneration in nutritionally anemic rats. They observed that hemoglobin regeneration was more rapid in the group receiving 20 ppm iron than in the group given 10 ppm iron. However, at either level of iron, dietary phytate had no effect on iron absorption as measured by total hemoglobin regeneration. These results could be due to the fact that rats have mucosal phytase in their gastrointestinal tract which will hydrolyze the iron-phytate complex and thus release iron for absorption (79,80,81). Also, the iron-phytate complex could be broken by the yeast phytase during the fermentation period or by the high level of phytase present in the wheat (82).

Based on an in vitro binding experiment in wheat flour, Reinhold et al. (83) concluded that fiber rather than phytate predominantly determines the availability of bivalent metal of bread for absorption by the intestine. These results explained why totally dephytinized wholemeal bread and bran showed an enhanced ability to bind metals. They believed that phytate is digestible. Like other digestible chelators, phytate would ultimately liberate bound metals when digestion progresses. On the other hand, metals bound by the indigestible fiber remain unavailable for absorption. Although fiber may be attacked by the large gut microbes, absorption can no longer occur in this region and the metals will be lost in feces. Incomplete digestion of phytate may interfere with the absorption of metals. Phytate would be important only to the extent that it escapes digestion.

Significance of phytase on phytate hydrolysis. Phytase is a nonspecific phosphomonoesterase (84). It catalyzes the hydrolysis of phytic acid to inositol and free orthophosphate.

Some properties of wheat phytase were studied by Peers (85). The optimal temperature was 55 C and the optimal pH was 5.15 for the hydrolytic activity of wheat phytase. This enzyme was relatively thermostable; it remained 85% active after 10 minutes at 80 C heat. Phytase activity was higher in the scutellum and aleurone fractions of wheat. Phytase activity of wheat increased about 6 fold on germination. Hard wheats contained more phytase and averaged about 20% more activity than soft wheat. The enzyme was partially inhibited by zinc and manganese salts. Calcium had no inhibitory action. The dialyzed enzyme was activated by Mg ions at a concentration of 0.002 M.

Phytate may be hydrolyzed which releases the bound minerals and leaves them free for absorption. Calcium and magnesium salts of phytate were hydrolyzed into inositol phosphoric acid, but ferric and aluminum salts were relatively inactive for hydrolysis (86). In studies of the mechanism of the hydrolysis reaction, Curtois and Masson (87) showed that phosphates were split off of alternate rather than adjacent hydroxyl groups in the early stages of hydrolysis.

Phytase occurs in the gastrointestinal tract of albino rats, quinea pigs, rabbits, pullets, chicks, and ruminants (88). Intestinal bacteria are known to produce phytase in these animals, but the production of phytase by intestinal flora in man has not been studied. Bitar and Reinhold (89) have found phytase activity in the first 12 inches of the human small intestine. Hydrolysis of phytate in the digestive tract depends on phytase from the walls of the digestive tract, microorganisms, and food itself. Ruminants utilize

phytate salt complexes better than nonruminants because they have phytase secreted by the rumen microorganisms (90).

Pringle and Moran (91) determined the effect of fermentation time on the destruction of phytate in doughs made with 85% extraction flour. They found that bread baked after 3, 5, and 8 hours of fermentation had 59, 64, and 76% hydrolysis respectively. The relatively high optimal temperature of phytase (85) should permit hydrolytic action to continue during the first stage of baking.

In an attempt to decrease phytate intake by introduction of yeast fermentation into breadmaking, Reinhold (92) described the experiments in which the effectiveness of yeast fermentation is compared in whole meals of different extraction rates. He found phytate was destroyed rapidly in whole meals of 75-85% and 85-90% extraction within 2 hours, but destruction was retarded in those of 95-100% extraction. The retardation persisted after 4 hours of fermentation and was still evident after 16 hours. Production of inorganic phosphorus kept pace with phytate destruction in the two whole meals of lower extraction rates but was delayed in those of 95-100% rate. The slow action of yeast phytase on the phytate of 95-100% extraction whole meals may be due to the presence of inhibitors. Such whole meals contain more calcium than do those of lower extraction. Calcium is believed to inhibit phytate hydrolysis by forming stable salts resistant to attack (93). High extraction whole meals also may contain more phytate in the protein-bound state than those of lower extraction rate. In addition, phytic acid itself in higher concentration inhibits phytase action (89,93).

To determine the rate and extent of decrease in phytic acid caused by sour-dough fermentation, Ter-Sarkissian et al. (94) measured the reduction in

phytic acid content during fermentation of dough commonly consumed in Iran. By lengthening the period of fermentation, the phytic acid content of the dough was decreased markedly. Increasing the fermentation time would seem to be the simplest method of reducing the phytic acid from bread, but longer fermentation increased the acidity of dough to the point where it was no longer acceptable. Significant reduction of the phytate level required the use of lower extraction flour. Hydrolysis increased substantially when yeast was added in increasing amounts. Increased hydrolysis was accompanied by increase in the level of available inorganic and residual phosphorus.

A chick study (95) was used to determine whether the addition of a mold phytase to a diet (corn and soybean meal) containing natural phytate phosphorus caused the phosphorus in the phytate to be hydrolyzed in vivo to a form which could be utilized. Addition of phytase to the diet produced an increase in percentage bone ash. Chicks utilized the hydrolyzed phytate phosphorus as well as supplemental inorganic phosphorus. Phytase activity was found in the alimentary tract of the chick but not in the feed prior to ingestion.

Possible absence (88) or inhibition (62,89) of intestinal phytase activity in human beings may be responsible for the high incidence of mineral deficiency disease in populations consuming high-cereal and thus high phytate diets. The ability of man to hydrolyze phytates in the digestive tract is probably due to microbial phytases (89) or nonenzymatic (gastric acidity) cleavage (96,97,98).

When Ranhotra and Loewe (99) fed rats corn starch (no phytase present) containing added phytate, the decrease in phytate phosphorus of ingesta indicated considerable phytate hydrolysis occurred in the stomach. Since no phytase is secreted by the rat stomach, this hydrolysis was due to wheat

phytase or nonenzymatical action. Rats fed phytase-inactivated wheat protein concentrate hydrolyzed appreciably less phytate in the stomach than those fed native wheat protein concentrate. This part showed that wheat phytase was involved in the phytate hydrolysis.

During fermentation with yeast, a proportion of phytic acid in the dough is hydrolyzed, the amount depending on the pH, temperature, length and humidity of the fermentation and the quantity of yeast used (100). More hydrolysis takes place in making white bread than in making brown bread, and with the latter it is more variable (101).

Ranhotra (102) stated that the quantity of yeast employed affects phytate cleavage somewhat. He suggested that 0.5%-3.0% yeast is suitable for hydrolysis. Excessive amounts have an reverse effect.

Widdowson (103) proved that baking bread with yeast led to considerable hydrolysis, and the more refined the flour, the greater the destruction. Eighty-five percent of the phytic acid in white flour (70% extraction), 69% of the phytic acid in national wheatmeal (85% extraction), and only 31% of the phytic acid in 92% extraction flour were destroyed during fermentation and baking of bread. When baking powder replaced the yeast, only 5% of the phytic acid was destroyed. Because baking powder such as sodium bicarbonate or cream of tartar was used, the dough was put in the oven as soon as it was mixed, which raised the temperature of the dough to a point at which the enzyme was destroyed. In addition, the optimal pH for wheat phytase is 5.15, but in the quick bread method the enzyme was never given a chance to react because of the alkaline condition created by the baking powder. When sodium phytate was added to white flour to make its phytic acid concentration equal to that in 92% extraction flour, more phytic acid was destroyed in baking than with 92%

extraction flour. The greater phytic acid destruction might be due to the fact that sodium phytate is more soluble than the phytates naturally occurring in the wheat.

Ranhotra (93) observed that when bread was made from 70% extraction white flour and 30% wheat protein concentrate, most of the hydrolysis occurred during the first 210 minute sponge time. Inorganic phosphorus increased as the phytate catabolized. Although phytase activity per se was not examined in this experiment, its activity is implied.

In a later experiment (102) Ranhotra reported that while phytic acid increased linearly with increasing amounts of wheat protein concentrate, phytase did not increase linearly. The amount of hydrolyzed phytic acid increased until 30% replacement with wheat protein concentrate was reached. Further replacement not only did not result in any increase in hydrolysis but the amount of phytic acid hydrolyzed was also inversely related to the replacement. The author's hypothesis was that decreased hydrolysis of phytic acid as wheat protein concentrate increased may be due to increased inhibition of phytase by change in pH or rephosphorylation (excessive accumulation of inorganic phosphorus) of partly hydrolyzed phytic acid.

MATERIALS AND METHODS

This study was conducted in two parts: (1) chemical determination of phytic acid in whole wheat flour and yeast-leavened whole wheat bread; (2) biological determination of the utilization of iron in whole wheat bread.

PREPARATION AND ANALYSIS OF YEAST BREAD

Basic procedure for bread baking. The 100% extraction hard red winter wheat flour used was obtained from the Dept. of Grain Science and Industry, Kansas State University. Three breads were made from yeast dough:

bread B - whole wheat bread made with live yeast, a three-hour bulk fermentation period, and a one-hour proof period (total fermentation of 4 hours).

bread C - whole wheat bread made with live yeast but no bulk fermentation or proof periods¹.

bread D - whole wheat bread made with dead yeast² and a four-hour standing period before baking.

The ingredients for the breads were:

whole wheat flour	1000 g
salt	10 g
sugar	60 g
shortening (Crisco)	40 g
yeast (compressed)	30 g
water (75 F)	600 ml

¹Steps 5-7 and 10 of procedure were omitted.

²The yeast suspension was boiled for 5 minutes to kill the yeast.

Steps in the basic procedure were:

1. Thirty grams of compressed yeast were weighed in a 250 ml Erlenmeyer flask. One hundred ml of 75 F water were added. The yeast was suspended thoroughly by using a magnetic stirrer for 5 minutes.
2. Flour, salt, sugar, shortening, yeast solution and the remaining 500 ml of 75 F water were put in a mixing bowl on a Hobart mixer (Model A-200).
3. The dough was mixed with a dough hook for 1 minute at the first speed; mixing was continued at the second speed until dough developed.
4. Two .539 g portions were scaled from the dough.
5. The scaled dough was transferred to a lightly greased shallow pan.
6. It was fermented for 2 hours at 85 F in a fermentation cabinet with a humidity of 86%.
7. The dough was taken out, punched down several times, and fermented for another hour.
8. The dough was dusted with flour and rolled with a moulder.
9. It was rolled into an oblong shape no bigger than the loaf pan. The edges were sealed by pressing tightly together.
10. It was placed in a loaf pan smooth side up and panproofed for 1 hour in the fermentation cabinet.
11. It was baked at 425 F for 25 minutes in a reel oven.
12. The bread was cooled at room temperature for 1 hour; then it was sliced; packaged and stored in a freezer.

Analysis of moisture, protein and iron of bread. Bread slices were spread out loosely on the racks of a ventilated cool oven and dried three days. They were ground finely by passing through a stainless steel grinder. The dried samples were analyzed for moisture by C.W. Brabender Semi-Automatic Rapid

Moisture Tester, for protein by macro-Kjeldahl method ($N \times 5.7$), and for iron by atomic absorption spectrophotometer after samples were wet-ashed in the Dept. of Agronomy. All analyses were conducted in duplicate. Average moisture, protein and iron of the breads are given in Table I.

TABLE I
Moisture, Protein and Iron of the Breads

	Bread B	Bread C	Bread D	Flour
Moisture (%)	6.2	6.0	6.3	10.2
Protein (g/100 g dry basis)	12.5	11.7	11.5	--
Iron (mg/100 g dry basis)	3.2	3.0	2.9	--

Analysis of phytic acid in flour and bread. The extraction and precipitation of phytic acid as ferric phytate were conducted according to Wheeler (44). The precipitated iron was measured by atomic absorption spectrophotometer. The phytic acid content was calculated assuming a constant 4 Fe : 1 phytic acid molecular ratio in the precipitate. The procedures were as described below:

1. Two 5 g samples of whole wheat flour and two 15 g samples of each kind of bread were put into 250 ml Erlenmeyer flasks.
2. Fifty ml of 3% TCA were used to extract the phytic acid by mechanical shaking for 1 hour.
3. The suspension was centrifuged for 20 minutes and then a 10 ml aliquot of the supernatant was transferred to a 50 ml plastic conical centrifuge tube.
4. Four ml FeCl_3 solution (made to contain 2 mg ferric iron per ml in 3% TCA) was pipetted into the aliquot.
5. The tube and contents were heated in a boiling water bath for 30 minutes. One or two drops of 3% Na_2SO_4 in 3% TCA were added. Heating was continued for another 15 minutes.
6. The solution was centrifuged for 15 minutes.
7. The supernatant was transferred to a 100 ml volumetric flask.
8. The precipitate was washed twice, each time with 20 ml of 3% TCA, heated in a boiling water bath for 10 minutes and centrifuged 10 minutes.
9. The wash was repeated once with demineralized distilled water.
10. All the supernatant from one sample was combined in a volumetric flask.
11. Iron contents of the supernatant and the stock solution, FeCl_3 were measured by atomic absorption spectrophotometry.

BIOLOGICAL DETERMINATION OF IRON UTILIZATION

Care of animals and diets. Forty weanling male Sprague-Dawley rats weighing 39 to 54 g were housed individually in stainless steel cages. Food and demineralized water were supplied ad libitum throughout the study. The basal diet was nutritionally adequate in all nutrients except iron (Table II). The basal and experimental diets were thoroughly mixed in a Hobart automatic mixer and stored at refrigerator temperature. The rats were fed the iron-deficient basal diet for 6 weeks at which time the average hemoglobin level of the animals was 9.4 g per 100 ml of blood. After the depletion period, five out of forty anemic rats were continued on the iron-deficient basal diet. The remainder was divided into 5 groups of seven rats each. These animals were assigned in such a way that the average hemoglobin level in every group was 9.4 g per 100 ml blood.

Dried breads were included in the regeneration diets (Table II) to provide 1 mg of bread iron per 100 g of diet. When bread was included in a diet, casein and dextrin were adjusted so that a constant level of protein and calories could remain in all diets. In control diet A, ferrous sulfate was added to the iron-deficient basal diet in an amount equivalent to 1 mg elemental iron per 100 g of diet. Breads B, C, and D were added to diets B, C, and D, respectively, so that each diet contained 1 mg bread iron per 100 g diet. Diet E had the same composition as diet C plus 1 mg of elemental iron in the form of ferrous sulfate.

The rats were kept on the regeneration diets for sixteen days. Food intake and weight gain were determined at the end of the experiment. Blood was obtained for hemoglobin and hematocrit determinations by amputating the tip of the tail of each rat.

TABLE II

Composition of Basal and Regeneration Diets

Ingredients (%)	Basal	Regeneration				
	F	A	B	C	D	E
Bread	--	--	33.3 ^c	35.7 ^d	37.0 ^e	35.7 ^d
Casein	13.05	13.05	8.73	8.69	8.63	8.69
Dextrin	40.0	40.0	12.52	10.16	8.92	10.16
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Vitamins ^a	2.2	2.2	2.2	2.2	2.2	2.2
Salt mix ^b	3.75	3.75	3.75	3.75	3.75	3.75
Cellulose	1.5	1.5	--	--	--	--
Sucrose	34.5	34.5	34.5	34.5	34.5	34.5
FeSO ₄ ·7H ₂ O	--	0.00496	--	--	--	0.00496

^aVitamin diet fortification mixture, Nutritional Biochemical Corp., Cleveland, Ohio.

^bModified Williams and Briggs salt mix with ferric citrate omitted(104).

^cBread B-- live yeast with 3 hours bulk fermentation, 1 hour proofing (total of 4 hours fermentation).

^dBread C-- live yeast, no bulk fermentation and no proofing.

^eBread D-- dead yeast with 4 hours standing before baking.

Hemoglobin determination. Hemoglobin was determined by the cyanmethemoglobin method (105). Six ml of cyanmethemoglobin reagent was pipetted into a colorimeter tube. A sample of 0.02 ml (20 μ) whole blood was added to each tube by using disposable micropipettes. The micropipette was rinsed 3 to 4 times with the reagent. A piece of parafilm covered the mouth of the tube during mixing. One tube was reserved as a blank to zero the Bausch & Lomb Spectronic 20 spectrophotometer. The tubes stood 10 minutes at room temperature before the absorption was read at a wavelength of 540 nm.

Hematocrit determination. Heparinized capillary tubes were filled with blood to 3/4 full. The end of the tube was sealed with plastic clay. The tube was centrifuged for 3 minutes in an Adams Autocrit centrifuge (CT-2905). Percentage of packed cell volume was determined from a hematocrit reading chart.

Statistical analysis. Data from weight gain, food intake, hemoglobin, hematocrit, and feed efficiency were subjected to analysis of variance. The analysis of variance was followed up with the use of Fisher's LSD with $P=0.05$ when the F-test rejected the hypothesis of equal treatment means.

RESULTS AND DISCUSSION

CHEMICAL EVALUATION. Phytic acid, phytic acid phosphorus, and the percentage of phytic acid hydrolysis in flour and the three whole wheat breads are shown in Table III. The mean phytic acid in the breads (expressed as mg per 100 g dry weight) was : 708 mg for bread B, 791 mg for bread C, and 763 mg for bread D. Fermentation with live yeast was a small factor in reducing phytic acid concentration. Bread B (4 hours fermentation with live yeast) produced a reduction of 38.4% phytic acid. Bread D (4 hours standing with dead yeast) led to a reduction of 33.6% phytic acid, whereas phytic acid in bread C (live yeast but no fermentation at all) was reduced 31.2%.

In bread C, a substantial amount of phytic acid was destroyed, showing that the major loss of phytic acid occurred during mixing of the dough and/or baking of the bread since there was no fermentation period. The slightly greater hydrolysis in bread B as compared to bread C was attributed to the function of yeast phytase plus wheat phytase during fermentation and baking. The destruction of phytic acid in whole wheat breads was little affected by yeast phytase alone as shown by a comparison of breads D and C.

Several investigators have claimed that the percentage of phytic acid destroyed during fermentation and/or baking decreased as the extraction rate of flour increased. In an in vitro experiment, Reinhold (92) compared the destruction of phytate by yeast fermentation in sponges prepared from Iranian whole wheat meals of different extraction rates. He found that phytate was destroyed rapidly in wholemeals of 75 to 85% and 85 to 90% extraction, but destruction was retarded in those of 95 to 100% extraction. Widdowson (103) reported that 31% of phytic acid in wheatmeal (92% extraction); 69% of phytic

TABLE III
Phytic Acid, Phytic Acid Phosphorus and Percent of Phytic
Acid Hydrolysis in Flour and Whole Wheat Breads

Sample	Phytic Acid (mg/100 g dry wt.)	Phytic Acid-P (mg/100 g dry wt.)	Phytic Acid Hydrolysis ^e (%)
Flour	1275 ^d	359	--
Bread B ^a	708	200	38.4
Bread C ^b	791	223	31.2
Bread D ^c	763	215	33.6

^a Live yeast with 3 hours bulk fermentation, 1 hour proofing (total of 4 hours fermentation).

^b Live yeast, no bulk fermentation and no proofing.

^c Dead yeast with 4 hours standing before baking.

^d Phytic acid in dough on a dry weight basis = $1275 \times \frac{100 \text{ (flour)}}{111 \text{ (dough)}}$

= 1149 mg/100 g dry dough

^e % hydrolysis = $\frac{\text{phytic acid in dough} - \text{phytic acid in bread}}{\text{phytic acid in dough}} \times 100$

acid in national wheatmeal (85% extraction), and 85% of phytic acid in white flour (70% extraction) was removed by yeast fermentation. Ranhotra (93) reported when bread was made from 70% extraction white flour and 30% wheat protein concentrate, there was a 43.4% decrease in phytic acid phosphorus after baking. Thirty-nine percent destruction of phytic acid was found by Ter-Sarkissian et al. (94) in village bread made of sifted whole wheat flour and fermented for a half hour.

The phytic acid phosphorus in Table III was calculated as :

$$\text{Phytic acid P} = \text{phytic acid} \times \frac{186 \text{ (M.W. of P in phytic acid)}}{660 \text{ (M.W. of phytic acid)}}$$

The flour analyzed in this study had a higher phytic acid phosphorus level (359 mg/100 g weight) than the range of 200-300 mg per 100 g basis in 100% extraction flour reported by Kent-Jones and Amos (106).

BIOLOGICAL EVALUATION. The analysis of variance for weight gain, food intake, feed efficiency, hemoglobin, and hematocrit is shown in Table IV. Diets had a significant effect on each of these factors ($P \leq 0.01$). Mean weight gain, food intake, feed efficiency, hemoglobin and hematocrit of rats on basal and yeast bread diets are shown in Table V. The values for individual animals are in the appendix (Table VII). Fisher's least significant differences at 5% level as well as 1% and 10% level (to be used with means in Table V) are shown in Table VI.

Weight gain. Control diet A produced an average daily weight gain of 5.3 g which was similar to the expected weight gain (5.5 g) for the first 53 days after weaning (107). Rats on diet E gained more weight than those on bread diets B, C, and D ($P \leq 0.05$), but not significantly more than those on diet A.

TABLE IV

Mean Squares and Significance^a for Weight Gain, Food Intake, Feed Efficiency, Hemoglobin and Hematocrit of Rats after Sixteen Days on Experimental Diets

Sources of Variation	Degrees of Freedom	Weight Gain	Food Intake	Feed Efficiency	Hemoglobin	Hematocrit
Diets	5	1489**	23836**	0.00292**	26.91**	271.4**
Rats:Diets	34	91.25	701.7	0.000671	1.328	6.640
Total	39					

^a **, $P \leq 0.01$.

TABLE V

Significance^a of Diet Effect on Mean Weight Gain, Food Intake, Feed Efficiency, Hemoglobin, and Hematocrit of Rats after Sixteen Days on Experimental Diets

Diet	Fe	No. of	Weight	Food	Feed	Hemoglobin	Hematocrit
	Source mg/100 g	Rats	Gain (g)	Intake (g)	Efficiency	(g/100 ml)	(%)
A	Ferrous Sulfate 1	7	84.5 ab	325.7 c	0.259 a	13.67 b	38.61 b
B	Bread B ^b 1	7	80.2 bc	336.0 bc	0.241 ab	13.39 bc	37.00 bc
C	Bread C ^c 1	7	72.4 c	360.1 ab	0.202 c	11.86 d	33.96 d
D	Bread D ^d 1	7	78.4 bc	353.3 abc	0.221 bc	12.38 cd	35.54 cd
E	Ferrous Sulfate + Bread C 2	7	93.3 a	376.6 a	0.248 ab	15.78 a	46.36 a
F	-- 0	5	45.5 d	193.3 d	0.236 ab	9.40 e	25.60 e

^aDiets with a common letter are not significantly different at $P \leq 0.05$.

^bLive yeast with 3 hours bulk fermentation, 1 hour proofing (total of 4 hours fermentation).

^cLive yeast, no bulk fermentation and no proofing.

^dDead yeast with 4 hours standing before baking.

TABLE VI

Fisher's Least Significance Difference at
the 10%, 5% and 1% Levels for Table V

Sample Size	Significance Level											
	Weight Gain			Food Intake			Feed Efficiency			Hemoglobin		
	10%	5%	1%	10%	5%	1%	10%	5%	1%	10%	5%	1%
5 & 7	9.5	11.4	15.3	26.2	31.5	42.3	.026	.031	.041	1.14	1.37	1.84
7 & 7	8.6	10.4	13.9	23.9	28.8	38.6	.023	.028	.038	1.04	1.25	1.68
										2.55	3.07	4.12
										2.33	2.80	3.76

There was no significant difference in weight gain among bread diets B, C, and D. Thus, whole wheat bread fermented for 4 hours produced no better rat growth than non-fermented whole wheat bread. Rats on the synthetic diet containing ferrous sulfate (diet A) grew more rapidly than those on whole wheat bread without fermentation (diet C) ($P \leq 0.05$). But there was no significant difference between diet A and bread diets B and D which provided the same amount of bread iron. Rats fed the iron-deficient basal diet F had the least weight gain which was significantly lower than any other diets ($P \leq 0.01$).

Food intake. Rats on diet E ate more food than those on diets A, B, and F ($P \leq 0.01$). There was no significant difference in the food intake of the three bread diets B, C, and D. Iron-deficient basal diet F resulted in the poorest food consumption of all the diets ($P \leq 0.01$).

The average daily food intake for control group A was 20.4 g which was close to the NRC requirement of 21 g (107). The amount of food consumed increased with decreased bread phytic acid (hydrolysis of phytic acid shown in Table III). Food intake by rats on diets A, B, C, and D had an inverse relationship to weight gain.

Feed efficiency. In order to compare the feed utilization of the various diets, feed efficiency was calculated as :

$$\text{Feed efficiency} = \frac{\text{weight gain (g)}}{\text{food intake (g)}}$$

The feed efficiency of the whole wheat bread diets tended to be less than that observed by Miller (108) in animals provided more bread iron (1.8 mg/100 g diet) than in the present study (1.0 mg/100 g diet). On her standard diet containing 1.14 mg Fe/100 g diet, she reported a feed efficiency of 0.293 which is slightly higher than 0.259 for control diet A containing 1.0 mg Fe/

100 g diet.

Feed efficiency of the basal and bread diets ranged from 0.259 for diet A to 0.202 for diet C. There was no significant difference in feed efficiency between diets E and A, although the former contained extra bread iron. Also there were no significant differences among diets A, B, E, and F. Iron-deficient basal diet F had the lowest weight gain and food intake but its feed efficiency was in the middle. The feed utilization of diet A was significantly higher than that of diets D and C. Apparently the rats could use bread B, which was higher in available iron, more effectively than bread C. The major difference between these two bread diets was that bread B contained a lower level of phytic acid than bread C ($P \leq 0.01$). Statistically there was no significant difference between bread diets B and D in feed efficiency.

Hemoglobin. Hemoglobin regeneration was used to evaluate the significance of the levels of phytic acid analyzed in the study. A level of 1 mg dietary iron per 100 g diet was considered submarginal for normal hemoglobin regeneration in the anemic rats. Therefore, in planning the present study, it was hypothesized that if phytic acid had any chelating potential, this level of iron might have a critical effect on iron absorption.

Rats fed diet E had an average hemoglobin level of 15.78 g per 100 ml blood which was above the mean value of 14.8 g per 100 ml blood (range 12.0-17.5 g per 100 ml blood) found in the Biological Data Book (109). The average hemoglobin level of rats on diet A, which provided a submarginal level of iron (1 mg), was 13.67 g per 100 ml blood. The hemoglobin concentration of diet E was significantly higher than that of diet A ($P \leq 0.01$). Therefore, it appeared that the existence of unbound iron from bread in diet E

had some benefit on hemoglobin regeneration.

Hemoglobin concentrations of rats on diets A and B were not significantly different. Therefore, iron of bread B which had undergone 4 hours fermentation with live yeast seemed to be used as well as iron in the form of ferrous sulfate. On the other hand, iron utilization of rats fed diets C or D was lower than when ferrous sulfate was fed. Hemoglobin concentration was higher ($P \leq 0.05$) for rats on diet B (fermented bread) than on diet C (unfermented bread).

The data shown in Table V indicated the regeneration of hemoglobin from iron in bread diets was dependent on their content of phytic acid (Table III). In the bread-containing diets, an increase in the absolute hemoglobin concentration after the repletion period occurred simultaneously with the increased hydrolysis of phytic acid. Apparently the hydrolysis of phytic acid made more iron available for absorption by anemic rats from bread B than from breads D and C. The influence of yeast phytase on iron availability to anemic rats did not reveal a remarkable effect on hemoglobin concentration when comparing results from diets D and C. This was expected since there was no appreciable difference in phytic acid hydrolysis between breads D and C. The combined action of yeast phytase and wheat phytase was evident in raising the hemoglobin level, since the average hemoglobin concentration for diet B was higher than that of diet C ($P \leq 0.05$).

Hemoglobin recovery was found to be significantly lower in the group receiving diet C than in that receiving diet E to which 1 mg extra iron was added ($P \leq 0.01$). The higher ($P \leq 0.01$) hemoglobin of rats on diet A relative to that of iron deficient basal diet F was primarily due to the addition of 1 mg iron. Hemoglobin of rats on the iron-deficient basal diet F during the

repletion period was as low as during the depletion period.

Hematocrit. Hematocrit is affected by iron absorption of animals although the response is not linear (16). The analysis of variance of hematocrit (Table V) was consistent with that obtained for hemoglobin concentration.

The mean hematocrit for rats given in the Biological Data Book (109) is 46% (range from 39 to 53%). Rats on diet E had an average hematocrit of 46.4% even though the iron of diet E (2mg/100 g diet) was less than the recommended 3.8 mg per 100 g diet for rats (107). Rats on diet E had a significantly higher hematocrit than rats on any other diets ($P \leq 0.01$). All the other diets produced comparatively low hematocrit values after the 16 day regeneration period. The hematocrit value of rats on diet A was significantly higher than those on diets D and C ($P \leq 0.05$), but there was no significant difference between diets A and B. Bread diet B produced a higher hematocrit than diet C ($P \leq 0.05$), but was not significantly different from diet D. Some inhibition of iron absorption in breads D and C was noted as both the hemoglobin and hematocrit values of rats on control diet A were significantly higher than those on diets D and C. This inhibition indicated that phytic acid in non-fermented or dead yeast-made bread may interfere with the availability of iron naturally occurring in the wheat.

CONCLUSIONS.

1. Chemical analysis of the phytic acid in the breads indicated that 31.2% of the phytic acid was hydrolyzed during mixing and the initial stages of baking of yeast leavened whole wheat bread. A 4-hour fermentation period only resulted in an additional loss of 7.2%, but this small loss apparently made significantly more iron available for hemoglobin regeneration in rats.

2. Both chemical analysis and hemoglobin regeneration studies indicated that wheat phytase was much more important in hydrolyzing phytic acid and making iron available to the body than was yeast phytase.
3. Anemic rats that are severely stressed for iron appeared to be able to use iron from yeast-leavened whole wheat bread containing substantial amounts of phytic acid as efficiently as iron from ferrous sulfate in a control diet.
4. Complete hemoglobin regeneration was not attained with 1 mg iron/100 g diet from either bread or ferrous sulfate; it was attained with 2 mg iron/100 g diet (1 mg from bread and 1 mg from ferrous sulfate).

The phytate in the bread did not prevent absorption of the additional iron from ferrous sulfate.

SUMMARY

The hydrolysis of phytic acid in whole wheat bread was determined by chemical analysis and the bioavailability of iron from whole wheat bread was assessed using anemic rats. Regeneration diets A, B, C, and D each supplied 1.0 mg iron/100 g diet which is considered to be submarginal. In diets B, C, and D the iron was provided by bread B (live yeast, 4 hrs fermentation), bread C (live yeast, 0 hr fermentation), and bread D (dead yeast, 4 hrs standing), respectively. Ferrous sulfate was added to iron-deficient basal diet F to make a control diet A. Diet E contained bread C plus 1 mg iron as ferrous sulfate/100 g diet for a total of 2.0 mg iron/100 g diet. After the 16 day repletion period, weight gain, food intake, hemoglobin concentration and hematocrit were measured and feed efficiency was calculated.

Yeast phytase together with wheat phytase resulted in 38.4% destruction of phytic acid during fermentation and baking of whole wheat bread B. The bread made with live yeast but without fermentation brought about a 31.2% reduction of phytic acid. Apparently this loss occurred during mixing of the dough and/or baking of the bread since there was no fermentation period.

Weight gain, food intake, feed efficiency, hemoglobin, and hematocrit were not significantly different for control diet A and bread diet B. Weight gain was higher but food intake was lower for control diet A than for bread diet C. Feed efficiency, hemoglobin, and hematocrit were significantly higher for control diet A than for bread diets C and D. Weight gain and food intake of rats fed bread diet B were not significantly different from those of the other two bread diets. However, feed efficiency, hemoglobin and hematocrit were significantly higher for bread diet B than for bread

diet C. Rats consuming bread diet C exhibited the lowest weight gain, feed efficiency, hemoglobin concentration and hematocrit among the bread diets. However, these values were highest for diet E which contained 1 mg elemental iron in addition to that supplied by the bread C formula.

It was concluded that wheat phytase was more important than yeast phytase in hydrolyzing phytic acid. Fermentation of bread increased the destruction of phytate and made more iron available for hemoglobin regeneration. Anemic rats utilized iron from fermented whole wheat bread as efficiently as iron from ferrous sulfate.

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APPENDIX

TABLE VII

Food Intake (g) and Initial and Final Body Weights (g), Hemoglobin (%),
and Hematocrit (%) for Different Diets Fed Sixteen Days

Diet	Description	Rat No.	Food Intake	Body Weight		Hemoglobin		Hematocrit	
				Initial	Final	Initial	Final	Initial	Final
A	Iron -	5	337	215	306	8.1	13.15	25	38
	Deficient	6	313	172	250	8.9	12.68	26	39
	Basal diet	19	324	178	266	9.8	14.43	27	39
	+ 1 mg Fe	20	319	170	251	9.4	14.13	26	39
		21	296	160	234	8.6	13.35	28	38
		25	367	192	286	10.2	14.65	26	40
		29	324	168	254	10.6	13.30	27	37
		2	361	180	256	7.9	13.10	24	38
B	Live yeast	9	318	130	196	9.7	12.65	28	37
	Fermentation	24	299	181	279	9.4	12.96	29	36
		27	342	149	229	8.5	13.15	24	35
		37	361	175	251	8.9	11.96	24	34
		39	317	132	208	10.2	13.45	28	36
		41	354	153	243	11.0	16.43	32	43

TABLE VII (cont'd)

Diet Description	Rat No.	Food Intake	Body Weight		Hemoglobin		Hematocrit	
			Initial	Final	Initial	Final	Initial	Final
C Live yeast	11	366	156	246	8.6	10.90	28	31
	15	337	163	231	10.0	12.47	32	35
	23	336	180	250	6.9	10.95	23	33
	30	442	157	233	13.0	12.88	31	37
	33	334	163	225	9.3	12.83	28	35
	35	367	178	251	8.3	11.15	20	32
D Dead yeast	38	339	159	227	9.5	11.85	28	34
	1	322	150	219	7.4	11.44	22	32
	3	354	177	259	9.3	14.33	31	36
	8	331	205	266	10.0	12.65	34	40
	13	368	153	245	9.5	12.64	28	38
	18	362	170	253	8.7	12.67	25	36
Standing	32	351	167	241	12.3	12.65	33	36
	34	385	190	278	8.4	10.30	23	31

TABLE VII (concluded)

Diets	Description	Rat No.	Food Intake	Body Weight		Hemoglobin		Hematocrit	
				Initial	Final	Initial	Final	Initial	Final
E	Diet C	4	377	174	267	9.4	14.83	29	44
		7	371	184	267	10.6	16.23	28	47
	+ 1 mg Fe	12	345	132	213	8.6	17.22	27	52
		16	385	159	261	10.2	16.72	33	45
		28	379	161	252	8.2	15.99	27	47
		36	402	163	275	9.0	14.27	27	45
		40	378	185	276	9.6	15.23	26	46
		14	204	170	225	9.3	10.27	25	25
		17	236	207	254	9.3	8.86	29	29
		22	169	160	196	9.7	10.65	29	27
		26	159	146	186	10.0	10.25	29	27
		31	199	172	222	8.5	6.99	23	20
F	Iron -								
	Deficient								
	Basal diet								

EFFECT OF PHYTIC ACID IN WHOLE WHEAT BREAD
ON IRON ABSORPTION

by

RUTHY LU-SZU WANG

B. Sc., National Taiwan University, Taiwan, 1974

AN ABSTRACT OF A THESIS

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The hydrolysis of phytic acid in whole wheat bread was determined by chemical analysis and the bioavailability of iron from whole wheat bread was assessed using anemic rats. Regeneration diets A, B, C, and D each supplied 1.0 mg iron/100 g diet which is considered to be submarginal. In diets B, C, and D the iron was provided by bread B (live yeast, 4 hrs fermentation), bread C (live yeast, 0 hr fermentation), and bread D (dead yeast, 4 hrs standing), respectively. Ferrous sulfate was added to iron-deficient basal diet F to make a control diet A. Diet E contained bread C plus 1 mg iron as ferrous sulfate/100 g diet for a total of 2.0 mg iron/100 g diet. After the 16 day repletion period, weight gain, food intake, hemoglobin concentration and hematocrit were measured and feed efficiency was calculated.

Yeast phytase together with wheat phytase resulted in 38.4% destruction of phytic acid during fermentation and baking of whole wheat bread B. The bread made with live yeast but without fermentation brought about a 31.2% reduction of phytic acid. Apparently this loss occurred during mixing of the dough and/or baking of the bread since there was no fermentation period.

Weight gain, food intake, feed efficiency, hemoglobin, and hematocrit were not significantly different for control diet A and bread diet B. Weight gain was higher but food intake was lower for control diet A than for bread diet C. Feed efficiency, hemoglobin, and hematocrit were significantly higher for control diet A than for bread diets C and D. Weight gain and food intake of rats fed bread diet B were not significantly different from those of the other two bread diets. However, feed efficiency, hemoglobin and hematocrit were significantly higher for bread diet B than for bread

diet C. Rats consuming bread diet C exhibited the lowest weight gain, feed efficiency, hemoglobin concentration and hematocrit among the bread diets. However, these values were highest for diet E which contained 1 mg elemental iron in addition to that supplied by the bread C formula.

It was concluded that wheat phytase was more important than yeast phytase in hydrolyzing phytic acid. Fermentation of bread increased the destruction of phytate and made more iron available for hemoglobin regeneration. Anemic rats utilized iron from fermented whole wheat bread as efficiently as iron from ferrous sulfate.