17. Lo, G.S., F.H. Steinke and D.T. Hopkins, J. Nutr. 110:829 (1980).
18. Rotruck, J.T., and K.R. Luhrsen, J. Agric. Food Chem. 27:27 (1979).
19. Steinke, F.H., and D.T. Hopkins, J. Nutr. 108:481 (1978).
20. Picciano, M.F., K.E. Weingartner and J.W. Erdman, Jr., (in preparation).
21. Layrisse, M., J.D. Cook, C. Martinez-Torres, M. Roche, I.N. Kohn, R.B. Walker and C.A. Finch, Blood 33:430 (1969).
22. Likuski, H.J.A., and R.M. Forbes, J. Nutr. 85:230 (1965).
23. Nahapetian, A., and V.R. Young, Ibid. 110:1458 (1980).
24. Nelson, T.S., T.R. Shieh, R.J. Wodzinski and J.H. Ware, Poult. Sci. 47:1842 (1968).
25. Tombs, M.P., Plant Physiol. 42:79 (1967).
26. Fontaine, T.D., W.A. Pons, Jr., and G.W. Irving, Jr., J. Biol. Chem. 164:487 (1946).
27. de Rham, O., and T. Jost, J. Food Sci. 44:596 (1979).
28. Okubo, K., A.B. Waldrop, G.A. Iacobucci and D.V. Myers, Cereal Chem. 52:263 (1975).
29. Omosaiye, O., and M. Cheryan, Cereal Chem. 56:58 (1979).
30. Likuski, H.J.A., and R.M. Forbes, J. Nutr. 84:145 (1964).
31. Oberleas, D., in "Proceedings of Western Hemisphere Nutrition Congress," edited by P.L. White and N. Selvey, Am. Med. Assoc., Chicago, IL, 1975, p. 156.
32. Davies, N.T., and S.E. Olpin, Brit. J. Nutr. 41:590 (1979).
33. Morris, E.R., and R. Ellis, J. Nutr. 110:1037 (1980).
34. deBoland, A.R., G.B. Garner and B.L. O'Dell, J. Agric. Food Chem. 24:804 (1976).
35. Ranhotra, G.S., R.J. Loewe and L.V. Puyat, J. Food Sci. 39:1023 (1974).
36. Morris, E.R. and R. Ellis, J. Nutr. 110:2000 (1980).
37. Martinez, W.H., in "Evaluation of Proteins for Humans," edited by C.E. Bodwell, AVI Publishing Co., Inc., Westport, CT, 1977, p. 309.
38. Van Stratum, P.G., and M. Rudrum, JAOCS 56:130 (1979).
39. Greger, J.L., R.P. Abernathy and O.A. Bennett, Am. J. Clin. Nutr. 31:112 (1978).
40. Sandström, B., and A. Cederblad, Ibid. 33:1778 (1980).
41. Janghorbani, M., and V.R. Young, Ibid. 33:2021 (1980).

Phytic Acid in Soybeans

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ABSTRACT

Phytic acid, the hexaphosphate of myo-inositol, is the most important phosphate reserve compound in many plant seeds, but many of its salts are poorly digested by animals. It can form complexes with seed proteins, some of which sequester metal ions, making them unavailable for the animal organism. Soya protein isolates may be higher in phytate content than the soya flour from which they are obtained. Zinc is the mineral of most concern because its bioavailability from some soya products is quite low and because of its marginal levels in some human diets. The availability of iron from soya flour and soya isolates is higher than that from some other plant foods with lower phytate contents. Processes for removing the larger part of the tightly bound phytates from soya protein isolates are described.

INTRODUCTION

Many plants contain chemical compounds with antinutritional properties. Some are probably produced as defense mechanisms against animal predators. Others are constituents of some physiological or mechanical importance for the plant, but are indigestible and even harmful for some animal species. Undoubtedly, there has been a long coevolution between plants and animals, during which the former developed enough defense mechanisms to be able to survive and the latter adapted their digestive and detoxification abilities to find enough food. It is therefore not surprising that most animal species are able to forage only on a limited number of plant foods (1).

Humans can consume a relatively wide range of vegetable materials. Nevertheless it has been estimated that even primitive tribes of hunter-gatherers exploit only a small part of the plant material available in their habitat, probably no more than 1-2%.

Even the vegetable foods we currently consume contain numerous ingredients that are not digested and absorbed or that are detoxified and excreted without direct physiological benefit for the eater. The final balance is important—whether one benefits more from ingesting certain foodstuffs or suffers more harm from it. Human intelligence, moreover, in many instances permits the elimination of the major harmful ingredients, thus making food available which would otherwise be inedible.

In the human diet, plant seeds play a dominant role. This is not surprising because they are the storage organs that provide nourishment for the developing young plant, while it is still incapable of photosynthesis and is therefore more similar in its physiological needs to an animal organism. These seeds quite often contain some defense constituent that may be toxic to animals. Their reserve materials are readily digested by their own enzymes during germination, but they are not always suitable substrates for the animal digestive enzymes. This is exactly the case of phytic acid, a reserve material in many plant seeds, which is not readily hydrolyzed in the digestive tract. Several excellent review articles have been published recently on the nutritional implications of phytates (2-5).

CHEMISTRY AND FUNCTION OF PHYTIC ACID

Phytic acid is the hexa-phosphate of myo-inositol and has three strongly bound water molecules. It can form a number of insoluble salts with different metal ions and can sequester several metals by chelate formation. Two or more cations, when present simultaneously, may have a synergistic effect and can act together to increase the quantity of metallic phytate precipitated.

Phytic acid is found mainly in plant seeds, where it functions as a reserve material for phosphorus. It is hydrolyzed during germination, when the phytase activity increases very rapidly. In cereals phytic acid is found mainly in the germ, which may contain 4-6% of the acid. Defatted soya meal contains about 1.5% phytic acid. Different fractions of oilseed meals may differ widely in their phytate content. During processing of soya isolates, various phytate-protein complexes are formed. At acid pH, phytic acid reacts with proteins, and upon neutralization, insoluble complexes are precipitated. These are still able to bind metal ions.

Mono-, di-, and tri-phosphoric esters of inositol exist in some phospholipids of animal tissues, but the hexaphosphate has been found only in plants.

DETERMINATION OF PHYTIC ACID

Most methods for the analytical determination of phytic acid take advantage of its property to form insoluble salts with iron in slightly acid solution. Coprecipitation of inorganic phosphate may be avoided by incubating the precipitate for 2 hr with 0.5 M HCl, as shown by Ellis et al. (6).

The precipitate may be hydrolyzed and the phosphoric acid determined quantitatively or the precipitated or excess iron can be measured colorimetrically, according to Davies and Reid (7) and Makover (8). After complete hydrolysis, the inositol moiety can be detected by thin-layer or paper chromatography.

IRON AND PHYTIC ACID

Most plant foods have poor iron availability for humans. The low solubility of ferric complexes with phytic acid lend weight to the assumption that this acid may be responsible for the significant differences in iron availability in animal and vegetable sources. There is now ample evidence that phytate is not a major factor.

Ellis and Morris (9) have shown that monoferric phytate is soluble under physiological conditions and is a good iron source for rats, whereas diferric and tetraferric phytate were poor sources. Practically nothing is known about the molecular form of iron in soya products. Davies and Reid (7) have presented analytical results showing that the iron content of soybean-based, textured vegetable protein (TVP) and commercial meat extenders varied considerably but were usually as high as that of raw soybeans. In rat studies the relative iron availability from soybean protein has been reported to be between 59 and 64% by Rotruck and Lursen (10). Steinke and Hopkins (11) compared hemoglobin repletion in rats fed soya protein isolates with that of rats fed ferrous sulfate. They found relative bioavailability of 61%. We have compared iron availability from iron-supplemented, degerminated corn meal with and without 8% defatted soya meal. The absorption from the latter was the same as that from the former (González and Jaffé, unpublished data). Iron from vegetable sources is usually much less absorbed in humans than in rats. In recent studies of Layrisse and Martinez Torres (unpublished data) with labeled iron added to different foods, absorption from soya meal in normal humans was 5%, compared with only 0.5% from polished rice, 2.5% from degerminated corn meal, 1% from wheat flour and 30% from calf liver. Evidently phytate cannot be the major factor in the poor iron availability of vegetable foods.

CALCIUM AND MAGNESIUM AND PHYTIC ACID

The action of phytate on the bioavailability of minerals was first studied with calcium when Mellanby (12) induced rickets by adding phytic acid to dog diets. This effect was ameliorated by vitamin D. In experiments performed by Momcilovic et al. (13) with rats, the absorption of Ca added to diets prepared with several soya products was the same as in the control casein diet. In a more recent paper, Taylor and Coleman (14) compared Ca absorption in rats and in the golden hamster in the presence of phytic acid. In both species absorption was impaired by increasing the dietary concentration of phytate. Phytate hydrolysis as measured by phosphorous balance was reduced when the dietary level of Ca was high. There were, however, important differences between the two animal species in their capacity to control absorption of both P and Ca. The traditional explanation for the interaction between Ca and phytic acid is that they form a highly insoluble salt and that phytase acts only on soluble phytates. The authors propose that the low-plasma Ca levels produced through low absorption of this mineral enhances parathyroid secretion and 25-hydroxycaliciferol-1-hydroxylase activity, thus compensating for the reduced Ca solubility.

Not much experimental work has been devoted to the study of the availability of Mg in the presence of phytate and of soya products. Lo et al. (15) used a slope-ratio method to measure Mg in blood serum and in femur bone of rats receiving varying amounts of Mg and crude or autoclaved, isolated soya protein, as compared with animals receiving diets of casein or of lyophilized beef. Statistical analysis of the results indicated that the protein sources did not have a significant effect on the bioavailability of Mg.

ZINC AND PHYTIC ACID

There is now strong experimental evidence that phytate may decrease zinc availability in animals and in humans. Bioavailability of this metal from soya protein isolate is much lower than from soya meal. The phytate-protein complexes formed during processing are probably responsible for the variability of zinc utilization in diets containing isolates manufactured by different processes (4).

Using a slope-ratio method to determine the availability of dietary efficiency of zinc by measuring weight gain and total femur zinc, Forbes and Parker (16) and Erdman et al. (17) found that, compared to other studied minerals, zinc was poorly utilized from soya products, but the presence of soya products had little effect upon the availability to the rat of added inorganic zinc. In the presence of high levels of calcium, insoluble Zn-Ca phytate complexes are formed (18). When soya-based meat extenders were fed to young rats they had lower growth rates and plasma Zn concentrations than the controls. Supplementation with Zn increased both the growth rate and plasma Zn level (7).

The phytate:zinc molar contents in experimental diets have been studied by Davies and Olpin (19) as possible indicators of zinc bioavailability in phytate-rich diets. However, some protein-phytate mineral complexes may be more active in reducing zinc absorption from some protein isolates than phytic acid alone. Dietary fiber, oxalates and phenolic compounds may also act on the absorption of dietary minerals.

In some phytate-rich soya products the Zn is poorly available to animals and this may have implications for human nutrition. Evidence has been presented of marginal Zn deficiency in populations of children in the U.S. (20). The average daily intake of Zn by middle-income group citizens of the U.K. falls short of the recommended requirements for adolescent males and females and pregnant and lactating women (7). The control of zinc availability from soya products is, therefore, of special interest.

ELIMINATION OF PHYTATE

Phytate is tightly bound to soya protein isolate. To partially eliminate phytic acid from soya products at a laboratory scale, several procedures have been used, such as precipitating as the barium salt, dialyzing against a sodium chloride solution, treating with a strong anionic exchange resin. Hartman (21) proposed the precipitation of phytate from soya protein by adjusting the solution at 28 C to pH 11.6. The precipitated phytates are then removed by centrifugation or by filtration. In the last step the extract is treated by ultrafiltration, resulting in a reduction of the phytate content from 2.6 to 0.1%. Using a pH of 5.5 and a Ca concentration of 0.0025 M, Ford et al. (22) prepared a soya curd that contained only 10% of the original concentration of phytic acid. De Rham and Jost (23) studied the solubility of phytate and protein in soya extracts as influenced by pH, NaCl, calcium and EDTA. Three processes for preparing low-phytate soya protein products were developed. The authors present a theory of the behavior of phytate in the presence of Ca, Mg, NaCl and soluble proteins.

The enzyme phytase exists in seeds and in many microorganisms. Soaking the seeds reduces the phytic acid content through enzymatic destruction, but the phytates in the mature dry seeds are very stable. During bread making, a considerable part of phytic acid is destroyed by enzymatic activity of the yeast. The fact that part of phytate phosphorous is available to animals shows that some phytate can be hydrolyzed in the intestinal tract, probably through the action of an alkaline phosphatase, which is activated by Mg ions (24).

Considerations for Edible Soya Products

To value the importance of the sequestering activity of phytic acid in edible soya products for human nutrition, one must consider the kind of product, the amount consumed and the kind of consumers. If the last are undernourished children or expecting or lactating mothers with low nutritional reserves and high nutritional requirements, evidently the effect may be different from that exerted on healthy, well-fed individuals. Soya products are frequently incorporated into products used for the treatment or prevention of malnutrition and are given in these cases for considerable lengths of time and in significant amounts. Possible mineral deficiency should be carefully considered, especially because Zn deficiency can produce anorexia.

Magee and Graininger (25) have recently shown that Zn added to low-protein rat diets is able to enhance growth significantly, suggesting that zinc supplementation could improve the apparent utilization of an inadequate level of dietary protein. At the same time, an antagonistic interrelationship between zinc and copper and zinc and iron at dietary zinc levels generally not considered to be toxic to young rats was evident by the marked decrease in liver copper and iron depositions in the presence of added zinc.

Whether it will be wise to supplement edible soya products with Zn or with a mixture of trace metals should be decided after careful considerations of the possible overall effects.

REFERENCES

1. Harborne, J.B., "Introduction to Ecological Biochemistry," Academic Press, New York and London, 1977, p. 58.
2. Erdman, J.W., Jr., JAOCS 56:736 (1979).
3. O'Dell, B.L., in "Soy Protein and Human Nutrition," Academic Press, New York and London, 1979, p. 187.
4. Rackis, J.J., and R.L. Anderson, Food Prod. Dev. 11:38 (1977).
5. Oberlease, D., in "Toxicants Occurring Naturally in Foods," NAS, Washington, DC, 1973, p. 363.
6. Ellis, R., E.R. Morris and C. Philpot, Anal. Chem. 77:536 (1977).
7. Davies, N.T., and H. Reid, Brit. J. Nutr. 41:579 (1979).
8. Makover, R.U., Cereal Chem. 47:288 (1970).
9. Ellis, R., and E.R. Morris, Nutr. Rep. Int. 20:739 (1979).
10. Rotruck, J.T., and K.R. Lursen, J. Agric. Food Chem. 27:27 (1979).
11. Steinke, F.N., and D.T. Hopkins, J. Nutr. 108:481 (1978).
12. Mellanby, E., J. Physiol. 109:488 (1949).
13. Momcilovic, B., Belonje, A. Giraux and B.G. Shah, Nutr. Rep. Int. 12:197 (1975).
14. Taylor, T.G., and J.W. Coleman, Brit. J. Nutr. 42:113 (1979).
15. Lo, G.S., F.H. Steinke and D.T. Hopkins, J. Nutr. 110:829 (1980).
16. Forbes, R.M., and H.M. Parker, Nutr. Rep. Int. 5:681 (1977).
17. Erdman, J.W., Jr., K.E. Weingartner, H.M. Parker and R.M. Forbes, Fed. Proc. 37:891 Abst. No. 3563 (1978).
18. Oberlease, D., M.E. Muhrer and B.L. O'Dell, J. Nutr. 90:56 (1966).
19. Davies, N.T., and S.E. Olpin, Brit. J. Nutr. 41:590 (1979).
20. Hambridge, K.M., P.A. Walvavens, R.M. Brown, J. Webster, S. White, M. Anthony and M.L. Roth, Am. J. Clin. Nutr. 29:734 (1976).
21. Hartman, G.H., JAOCS 56:734 (1979).
22. Ford, J.R., G.C. Mustakas and R.D. Schmutz, Ibid. 55:371 (1978).
23. De Rham and T. Jost, J. Food Sci. 44:596 (1979).
24. Pileggi, V.J., Arch. Biochem. Biophys. 80:1 (1959).
25. Magee, A.C., and F.P. Graininger, Nutr. Rep. Int. 20:771 (1979).

Significance of Soya Trypsin Inhibitors in Nutrition

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ABSTRACT

Although recent evidence clearly indicates that trypsin inhibitors (TI) and low protein digestibility are the major factors responsible for the pancreatic hypertrophic and growth inhibitory effects of raw soybeans, there was uncertainty regarding the biological threshold level of TI at which these biological effects occur. To obtain such data, dehulled defatted flakes (10% dietary protein) containing graded levels of TI were fed to weanling rats for 4 weeks in two feeding trials. Normal pancreas weights were obtained in rats fed samples in which only 54 to 68% of the original TI of raw soya flour was inactivated. In partially toasted flakes with a nitrogen digestibility value of 77%, the average tolerance level of dietary TI activity that did not cause pancreatic hypertrophy was calculated to be 385 mg TI/100 g diet. TI tolerance level at maximum nitrogen digestibility of 85%, which did not significantly lower weight gain and reduce protein efficiencey ratios, was 260 mg TI/100 g diet. Continuous ingestion of high levels of TI (459 mg TI/100 g diet) in a 20% protein diet for 215 days did not inhibit growth nor cause pancreatic hypertrophy when compared to rats fed toasted soya flour diets. Pancreatic hypertrophy that occurs in rats fed raw soya diets containing up to about 1300 mg TI/100 g diet for 35 days was reversed by switching the rats to control diets or to 30% toasted flour. In long-term feeding studies, no pancreatic hypertrophy occurred in rats fed commercial edible-grade soya flour, concentrate, or isolate from time of weaning to adulthood (ca. 300 to 330 days). TI content of the diets ranged from 178 to 310 mg/100 g diet. Microscopic examination of the pancreas revealed no abnormalities. Gross appearances of heart, kidney, spleen and liver were normal. In long-term feeding, vitamin B-12 supplements were needed to provide optimum growth and to maintain body weight. Results of numerous chemical analyses, relatively short-term human tests and long-term animal feeding studies indicate that with proper control of manufacturing processes, soya protein products can be produced that, in mixed diets, have protein nutritional value approaching that of animal protein.