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SOY PROTEIN AND HUMAN NUTRITION

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EFFECT OF SOY PROTEIN ON TRACE MINERAL AVAILABILITY[1]

Boyd L. O'Dell

INTRODUCTION

With the increasing use of soybean protein in the human diet and its substitution for animal protein, the importance of assessing its nutritional properties is evident. Assessment of its contribution to trace element nutrition involves not only estimation of trace element content but also evaluation of bioavailability. Bioavailability is defined here as that proportion of a chemically determined nutrient which is absorbed and utilized. Such an assay must involve an animal and finally man himself.

I. *Bioavailability and Its Evaluation*

Absorption and utilization of any given element depends on such intrinsic factors as species and the physiological state of the animal, but this discussion will be restricted to the extrinsic or dietary factors that affect bioavailability. Extrinsic factors that may affect bioavailability include:

a) Chemical nature, or speciation of the element.
b) Competitive antagonism among closely related elements.
c) Adsorption on large surfaces of insoluble compounds.
d) Chelate or complex formation; this effect may be detrimental or beneficial.
e) Microbial flora in the gut.

This discussion is further restricted to those factors associ-

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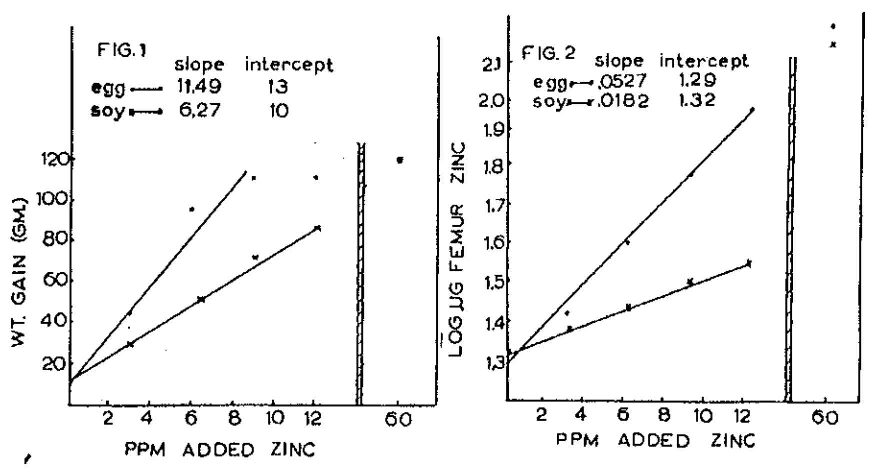
ated with soybean protein which may influence bioavailability. For a more general treatment of the subject see an earlier review (O'Dell, 1972).

Various bioassays have been used to measure bioavailability of trace elements, but only iron and zinc have received major attention. In human nutrition, mineral balance or net retention of an element is commonly employed, except for iron, where the use of isotopes is more popular (Sharpe et al, 1950). The regeneration of hemoglobin in iron-depleted animals in also used (Fritz et al, 1970). For the evaluation of zinc bioavailability, growth rate of animals fed limiting levels of zinc (O'Dell, 1969 and O'Dell et al, 1972a) and total femur zinc (Momcilovic et al, 1976) have been employed. Forbes and Parker (1977) used slope ratios of both gain and total femur zinc in rats to evaluate zinc availability in full fat soy flour. Their results are shown in Figures 1 and 2. The weight gain data give a slope ratio of 54% compared to 34% for the femur zinc data. Which is the more reliable value is a moot question, but theoretically it would seem more valid to consider zinc accretion during the assay than total zinc. Such a treatment of the data gives a higher availability for the soy protein and one in better agreement with the growth data. In addition to the methods described, both intrinsically (Welch et al, 1974) and extrinsically (Evans and Johnson, 1977) labeled zinc have been used to evaluate zinc absorption.



FIGURES 1 & 2. Use of slope ratios of weight and total femur zinc to evaluate bioavailability of zinc in whole fat soy flour compared to zinc carbonate added to egg white protein. Taken from Forbes and Parker (1977).

II. *Composition of Soybeans and Products*

The major soybean products used for human food are defatted soy flour, protein concentrates and protein isolates. The concentrates are prepared by leaching with alcohol, acid or water to remove some of the carbohydrates. The protein isolates consist of the water soluble proteins and are nearly free of carbohydrate. Selected values of the gross compositions are shown in Table 1. For the purpose of this discussion the most important constituents are crude fiber and phosphorus. Calcium content is of secondary interest. Approximately 70% of the total phosphorus in soybeans and soybean protein products occurs in the form of phytate, inositol hexaphosphate. It should be pointed out that phytate could not occur, as is commonly stated, solely as phytin, Ca_5 Mg Phytate, because there is not sufficient calcium to account for this compound. This is certainly the case for the isolated protein which contains low concentrations of both Ca and Mg. Even whole soybeans contain less than 3 g atoms of Ca per mole of phytate.

Soybeans contain a complex mixture of carbohydrates which make up about one third of the mass. Of this about 5% may be classed as crude fiber. However, isolated soy protein contains no fiber.

Soybean trace elements of nutritional, or possible nutritional, significance are shown in Table 2. Of the 15 elements listed, there is little doubt about the nutritional significance of the first nine. The significance of some of the others is now under study. Nevertheless, from a practical standpoint Fe and Zn are of primary concern in human nutrition and the only ones for which appreciable bioavailability data have accrued.

Since phytate is the constituent of seeds of greatest concern relative to bioavailability of the trace metal cations, it is of interest to compare the phytate concentration in various products. The phytate content of selected seeds and plant proteins are shown in Table 3. Sesame and rapeseed meals have the highest phytate concentration with soybeans ranking higher than the cereal grains. In the case of corn, essentially all of the phytate is in the germ, while 87% of the phytate in wheat is in the aleurone layer and 80% of that in rice is in the pericarp (O'Dell et al, 1927b). Phytate is usually determined by precipitation with ferric ion in acid solutions. This reagent will precipitate inositol phosphate esters containing less than six phosphates. However, only the hexaphosphate of inositol was detected in mature corn, wheat, rice, sesame and soybean (Boland et al, 1975).

Table 1. *Major Constituents of Soybeans and Soybean Products (Dry Basis)*

	Whole Soybean[1]	*Soy Flour*[2]	*Soy Concentrate*[3]	*Isolated Protein*[2,3]
	%	%	%	%
Protein	42.9	51.6	71.6	98.7
Fat	19.6	0.93	0.37	-
Ash	5.0	-	6.5	3.2
Crude Fiber	5.5	2.8	3.8	0.2
NFE (Difference)	27.0	-	17.7	-
K	1.7	2.3	2.34	0.096
Ca	0.3	0.23	0.25	0.18
Mg	0.3	0.28	0.29	0.038
P	0.7	0.75	0.66	0.76
Phytate P	0.5	-	-	0.53

[1]*Cartter and Hopper (1942).*
[2]*Lo (1978).*
[3]*Meyer (1978).*

Table 2. Trace Element Content of Soybeans and Isolated Soy Protein[1]

Element	Defatted Soybeans[2]	Soy Protein[3]
	ppm	ppm
Fe	137	160
Zn	52	40
Cu	20	12
Mn	38	17
Cr	0.35	1
Mo	2	3
I	0.84	-
F	1.9	-
Se[4]	0.065	0.137
Si	140	-
Ni	6	2.5
V	0.04	10
As	0.04	-
Li	0.1	0.023[5]
Co	0.24	1

[1]Selected values for elements of nutritional or possible nutritional significance, dry basis.
[2]Osborn (1977) except Se.
[3]Lo (1978) except Se and Li.
[4]Se from Ferretti and Levander (1976).
[5]Patt and Pickett, personal communication.

Table 3. Phytate Content of Selected Seeds and Proteins[1]

Product	Phytate P
	%
Corn, commercial	0.25
Corn, high lysine	0.28
Wheat, soft	0.32
Rice, brown	0.25
Sesame meal, defatted	1.46
Rapeseed flour[2]	1.12
Pea seeds, mature[3]	0.33
Beans (Phaseolus vulgaris)[4]	0.18-0.39
Soybeans (15 varieties)[5]	0.28-0.41
Soybean meal, comm.	0.40
Soybean flakes, defatted	0.43
Isolated soy protein, comm.	0.43

[1]*Boland et al (1975) except as noted.*
[2]*Anderson et al (1976).*
[3]*Welch et al (1974).*
[4]*Lolas and Markakis (1975).*
[5]*Lolas et al (1976).*

III. Bioavailability of Zn, Fe, Cu, Mn and Mg in Soybean Protein

Zinc. The first observations that soybean protein affects trace element availability arose from zinc requirement studies. Chicks fed soybean protein had a higher zinc requirement than those fed casein and gelatin (O'Dell and Savage, 1957). It was soon shown that either autoclaving the soy isolate or addition of ethylenediaminetetracetate (EDTA) reduced the zinc requirement of turkey poults, pigs and rats fed diets based on soy protein (Kratzer et al, 1962; Smith et al, 1962; Forbes, 1964). In the latter study a strong interaction was observed between calcium and soy protein as regards zinc requirement. Observations similar to those made with soy protein have been made with sesame meal (Lease et al, 1960) and rapeseed flour (Anderson et al, 1976). Although less of the zinc in soy protein is absorbed and utilized than of that in animal protein, this fact may not be of practical importance when there is only partial substitution of soy for meat protein in the human diet. Greger et al (1978) substituted rehydrated defatted soy (Textured Vegetable Protein), for 30% of the meat in the lunch of adolescent girls and found no effect on fecal or urinary zinc loss.

A few attempts have been made to quantitatively assess the biological availability of zinc in plant and animal foodstuff. Values relative to zinc salts, such as $ZnCO_3$ or $ZnSO_4$, are shown in Table 4. In general the zinc in animal products is more available than that in plant products, soybean zinc being 50-65% available. It has been postulated that phytate accounts for the low bioavailability of zinc in seed proteins (O'Dell and Savage, 1960). The effect of phytate will be discussed below.

Iron. It is generally concluded that the iron, particularly heme iron, in animal foods has a higher bioavailability than that in plant foods (Bowering et al, 1976). Based on this generalization one would expect the iron in soybean products to be poorly utilized. In confirmation of this concept Fitch, et al (1964) observed a lower absorption of iron in Rhesus monkeys fed soybean protein than those fed casein. However, bioavailability assessment by hemoglobin repletion in the rat suggests a relatively high availability. Steinke and Hopkins (1978) observed an average of 61% bioavailability of the iron in isolated soy protein.

Similar high iron availability was observed in the growing chick (Davis et al, 1962). Whereas the zinc in isolated soy protein was poorly utilized unless EDTA was added, there was no effect from adding EDTA when iron was limiting. Considering the fact that ferric iron forms highly insoluble complexes with phytate, one might expect a low availability of iron in foods rich in phytate. Soybean protein is relatively high in both iron and phytate but the chemical form of the iron is unknown. Morris and Ellis (1976) have characterized a major iron component of wheat bran as monoferric phytate and showed that this soluble iron salt is as available as ferrous ammonium sulfate. Although phytate may have an effect on iron availability, it seems clear that phytate is not the only factor involved. Welch and van Campen (1975) labeled soybeans with ^{59}Fe and administered single oral doses of immature and

Table 4. Bioavailability of Zinc in Feed and Foodstuffs

Product	Assay Animal	Method	Value
			%
Milk based infant formula	Rat	Slope ratio-femur	86[1]
Soy based infant formula	Rat	Slope ratio-femur	67[1]
Whole fat soy flour	Rat	Slope ratio-femur	34[2]
Whole fat soy flour	Rat	Slope ratio-gain	54[2]
Soybean meal	Chick	Growth rate	67[3]
Sesame meal	Chick	Growth rate	59[3]
Corn	Chick-rat	Growth rate	63,57[3]
Wheat	Chick-rat	Growth rate	59,38[3]
Rice	Chick-rat	Growth rate	62,39[3]
Egg yolk	Chick-rat	Growth rate	79,76[3]
Non-fat milk	Chick-rat	Growth rate	82,79[3]

[1]*Momcilovic et al (1976).*
[2]*Forbes et al (1977).*
[3]*O'Dell et al (1972).*

mature seeds to rats. Approximately 32% of the iron in the immature seeds, which contained 0.61% phytic acid, was absorbed compared to approximately 52% in the mature seeds, which contained 1.71% phytic acid. Apparently the immature seeds contain a factor other than phytate which impaired iron absorption. However, one must recognize that absorption of an ion from a single dose may be different from that in a diet consumed *ad libitum*.

Copper, Manganese and Magnesium. There is little information in the literature relative to the bioavailability of these elements. As mentioned above, addition of EDTA to a soy protein based diet increases the availability of zinc. Davis, et al (1962) also observed that the addition of 0.07% EDTA im-

Foodstuffs

	Value
	%
io-femur	86[1]
io-femur	67[1]
io-femur	34[2]
io-gain	54[2]
te	67[3]
te	59[3]
te	63,57[3]
te	59,38[3]
te	62,39[3]
te	79,76[3]
te	82,79[3]

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proved the response of chicks fed soy protein when either copper or manganese was limiting. EDTA had no effect when these elements were adequate. On this basis it was concluded that soybean protein binds Zn, Cu and Mn and makes them less biologically available.

Strictly speaking, magnesium is not a trace element, but there is some evidence that phytate decreases its absorption. If so, one might expect soy protein to decrease Mg availability. Two groups (Erdman et al, 1978 and Lo et al 1978) have recently examined this question and concluded that soy protein has no significant effect on Mg availability.

IV. Constituents of Soybean Protein that may affect Bioavailability

Phytate and Zinc. Phytate phosphorus makes up approximately 70% of the total phosphorus in soybeans and it is associated with the protein in soybean products, including the isolated protein. There is overwhelming evidence that soluble phytate added to purified diets decreases zinc availability in chicks (O'Dell and Savage, 1960; Likuski and Forbes, 1964), pigs (Oberleas et al, 1962), rats (Oberleas et al, 1966; Likuski and Forbes, 1965) and man (Reinhold et al, 1973). Not only does phytate bind zinc and make it less readily absorbed but, as in the case of soybean protein, excess calcium aggravates the situation (Oberleas et al, 1962; O'Dell et al, 1964; Likuski and Forbes, 1965; Oberleas et al, 1966). In the absence of phytate excess calcium has no effect on zinc availability. It has been postulated that calcium, zinc and phytate interact to form a highly insoluble complex which reduces the absorption of zinc to a greater extent than phytate alone (Byrd and Matrone, 1965; Oberleas et al, 1966). The addition of EDTA to diets containing soluble phytate increases the absorption of zinc (Oberleas et al, 1966; O'Dell et al, 1964) just as it does when added to soy protein. Data illustrating the interaction of calcium, zinc and phytate as well as the counteracting effect of EDTA are shown on Table 5. From these and similar data, it is clear that absolute values of bioavailability cannot be obtained. Bioavailability of zinc depends, among other things, on the presence of chelating agents and on the calcium concentration in the diet.

As regards the effect of phytate on zinc availability, it is important to consider the molar ratio of phytate to zinc, taking into account the calcium to phytate ratio as well. From the data presented in Table 5, it appears that 8 ppm of zinc is adequate for the rat if the diet contains no phytate, but it is grossly inadequate in the presence of 1% phytate. Such a diet would have a phytate:Zn ratio of 123:1.

Table 5. Effect of Calcium-Zinc-Phytate Interaction and of EDTA on Zinc Availability in Rats

Dietary Variables			Weekly Gain, g	
Ca	Phytate	EDTA	Zn Supplement	
%	%	%	0	55 ppm
0.8	-	-	32	31
1.6	-	-	33	32
0.8	1.0	-	20	31
1.6	1.0	-	7	33
1.6	1.0	0.1	23	-
1.6	-	0.1	26	-

Data adapted from Oberleas et al (1966). Diet was based on casein and contained approximately 8 ppm Zn. Weanling rats were fed the diets for 4 weeks.

Although there are insufficient data to establish a tolerable ratio, it is probably less than 20:1 (Ellis and Morris, 1978). Zinc phytate itself is well utilized (Green et al, 1962; Ellis and Morris, 1978). In this regard it is significant to point out that high levels of phytate in the diet reduce the biological half-life of zinc in the rat, possibly by inhibiting resorption of endogenous zinc and thus promoting loss of zinc other than that associated with phytate in the diet (Davies and Nightingale, 1975).

Phytate and Iron. There are conflicting results regarding the effect of phytate on iron bioavailability. McCance et al (1943) observed that addition of sodium phytate to white bread decreased iron absorption and they attributed the lower iron balances of persons consuming brown bread to its higher phytate content. In a study with adolescent boys, Sharpe et al (1950) observed that addition of sodium phytate to milk (0.2 g in 200 ml) decreased iron absorption 15-fold. However, the same quantity of phytate in the form of oatmeal had much less effect. In a study with rats, Davies and Nightingale (1975) found that addition of 1% phytate to an egg albumin diet, significantly reduced the whole body retention of iron as well as of zinc, copper and manganese. Others have found little or no adverse effect of dietary phytate on iron utilization (Fuhr and Steenbock, 1943; Cowan et al, 1966; Ranhotra et al, 1974). Morris and Ellis (1976) have found that the iron of monoferric phytate is well utilized, but that insoluble ferric phytate has a much lower bioavailability. These conflicting results suggest that different and undefined conditions existed in the various laboratories. It is possible that phytate interacts with proteins endogenously or when it is added to certain diets. Under conditions of protein-phytate interaction, iron may not be bound efficiently. This concept is supported by the observation of Sharpe et al (1950) that when added to milk the phytate in rolled oats did not prevent iron absorption as effectively as sodium phytate.

Fiber and Zinc. Several studies suggest that phytate is not the only constituent of plant foods that adversely affects zinc utilization. Reinhold et al (1973) observed that unleavened whole wheat (Tanok) bread had a more detrimental effect on zinc balance than sodium phytate supplied daily at the same level as that in bread. However, it should be noted that the phytate was supplied in orange juice separate from the leavened bread. Although this observation could be explained by the chemical form of the phytate, e.g., protein-phytate complexes, there is evidence that fiber decreases absorption of zinc, calcium, magnesium and phosphorus (Reinhold et al, 1976; Ismail-Beigi et al, 1977). In the latter study 10 g of cellulose decreased the retention of these elements when added to a diet that supplied 500 g of bread containing 0.35% of phytate and 3.6% of crude fiber. Addition of cellulose increased the stool size and, no doubt, the rate of passage through the intestinal tract. Whether these changes explain the decreased retention of the elements or there is specific binding to cellulose or other dietary fiber components is not clear. Sandstead et al (1978) tested five sources of dietary fiber, including wheat bran, and concluded that fiber did not alter zinc balance in adult men. Guthrie and Robinson (1978) performed zinc balances in four young women, with and without daily supplements of wheat bran (14 g/day), and found no overall difference in zinc metabolism. Ellis and Morris (1978) found that removal of phytate from wheat bran eliminated its detrimental effect on zinc bioavailability even though the dietary fiber content was unchanged. Caution must be exercised in drawing conclusions relative to the effect of dietary fiber on trace element availability because "dietary fiber" is ill-defined and difficult to determine.

V. *Phytate-Protein Interaction*

Phytate forms strong complexes with some proteins and such complexes are less subject to proteolytic cleavage than the free protein (O'Dell and Boland, 1976). Calcium also interacts with phytate and protein to decrease solubility. In view of these facts it is conceivable that protein-phytate complexes bind zinc and other cations more tenaciously than phytate alone. However, it is clear that protein is not essential for phytate to decrease zinc utilization. Likuski and Forbes (1964) observed that phytic acid decreases the bioavailability of zinc as effectively when amino acids serve as the nitrogen source as when casein is present.

Nevertheless, we have examined extracts of corn germ, sesame meal and defatted soybeans for protein-phytate complexes. Boland et al (1975) observed that the phytate in corn germ is highly water soluble (pH 6.1), that in sesame meal is only slightly water soluble (pH 6.8), while essentially none of that in isolated soy protein is soluble in water (pH 4.9). The distribution of phytate and protein in fractions of defatted soybean flakes is shown in Table 6. Under the conditions used, water extracted approximately 60% of both protein and phytate. Adjustment of the water extract to the isoelectric point (pH 4.5) precipitated slightly more than half of the protein and phytate. Most of the phytate left in the supernate was lost by dialysis.

Table 6. Protein and Phytate Content of Defatted Soybean Fractions

Fraction	Solids	Crude Protein	Phytate P Conc.
	g	g	%
Original Flakes	100	49.7	0.43
H_2O Sol.	38	29.4 (59%)[1]	0.68 (60%)[1]
Residue	60	15.7 (32%)	0.14 (20%)
Isoelect. Ppt of H_2O Sol.	20	18.1 (36%)	0.66 (30%)
Isoelect. Sol. (Dialyzed)	11	1.9 (3.7%)	0.35 (9%)

[1]*Percentage of original recovered.*

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	Phytate P Conc.
	%
	0.43
	0.68 (60%)[1]
	0.14 (20%)
	0.66 (30%)
	0.35 (9%)

To determine protein-phytate interactions, duplicate samples of the fractions were electrophoresed on polyacrylamide gels at a running pH of 9.3. One gel was stained for protein with amidoschwarz and the other reacted with $FeCl_3$ to detect phytate. Corresponding bands indicate protein-phytate interactions. Figure 3 shows gels of the fractions prepared from soybean flakes and Figure 4 gives the density profile of the proteins in the original water extract. Phytate was associated with at least three protein bands and a large proportion appeared at the buffer front. The major protein band, second from the origin, contained appreciable phytate as did a band which moved near the front. The latter band appeared in the isoelectric supernate and represents either low molecular material or protein with a high negative charge. The isoelectric precipitate contained one band of phytate which was associated with the major protein near the origin. Figure 3 also shows similar data for sesame meal.

FIGURE 3. Polyacrylamide gel electrophoretograms of defatted soybean extracts. The left one of each pair was stained with amidoschwarz to detect protein and the right one was stained with $FeCl_3$ to detect phytate. The first pair on the left was the total water soluble extract, the center pair, the isoelectric precipitate and the right hand pair the supernate of the isolectric precipitate.

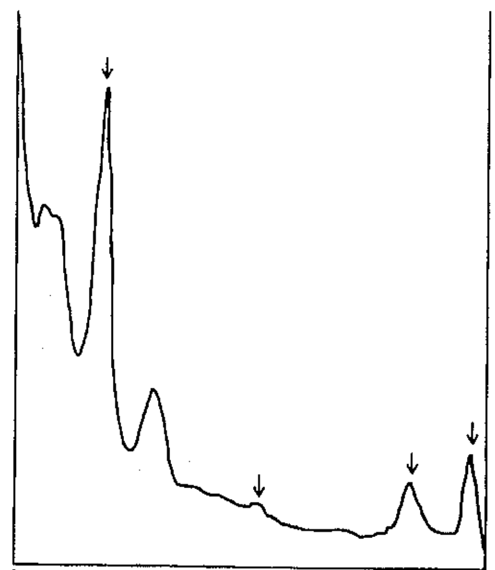

FIGURE 4. The density profile of the water soluble proteins of defatted soybean flakes; gel on the left of Figure 3. O corresponds to the origin and F the buffer front of the gel. The arrows indicate protein bands that correspond with phytate bands in the duplicate gel.

The effect of protein-phytate complexes on zinc bioavailability has not been tested. In fact, it is not known whether such complexes exist in seeds or are simply artifacts of preparation. In order for protein-phytate complexes to decrease zinc absorption more than phytate alone, they would have to be indigestible. Against this concept is the observation that the percentage of fecal nitrogen in the chick was not different when soy protein and casein were fed (Savage et al, 1964). However, a relatively small phytate peptide complex might be highly effective in metal binding. While this unresolved problem is of interest, it appears that protein-phytate complexes are of little nutritional significance.

VI. *Possibilities of Reducing Phytate in Foods*

Soy protein is an excellent source of amino acids for human and animal nutrition. One limitation to its wide use is its propensity to bind zinc and decrease zinc absorption. The phytate associated with soy protein is the major contributor to zinc binding, and it appears that elimination of this component would improve the value of soy protein as a human food. This might be accomplished by genetic selection or chemical treatment. An approach to selection of wheat varieties low in phytate has been made by Bassiri and Nahapetian (1977) and a similar attempt might be made with soybeans. A more immediate solution might be achieved by removal of phytate from soy products through the action of phytase, an enzyme that cleaves the phosphate ester bonds of phytate. Reinhold et al (1974) were able to reduce the phytate content of bread minimally by use of yeast, but there appears to be an inhibitor in whole wheat. Soybeans contain little or no endogenous phytase activity. However, Ranhotra et al (1974) found that at least 75% of the original phytate was hydrolyzed during the process of making bread from flour enriched with 10% soy flour. Maximal hydrolysis occurred when 9 g of yeast, the customary amount used, was added per pound loaf. Phytate can be removed by treatment of the water soluble proteins of defatted soybeans with an anion exchange resin (Smith and Rackis, 1957) or by autoclaving (Boland et al, 1975), but neither of these methods appears to be of practical importance. By selection of the conditions of precipitating soy protein lower than usual phytate content can be achieved (Ford et al, 1978). Phytate removal was best attained by either low pH (3.5-4.0) and high calcium (0.04 M) or a higher pH (5.0-5.5) with low calcium. Using a pH of 5.5 and a calcium concentration of 0.0025 M, Ford et al (1978) prepared a curd from full-fat soy flour which contained 0.04% phytate phosphorus, only 10% of the original concentration.

CONCLUSIONS

Among the essential trace elements of practical importance in human nutrition, only zinc has been clearly shown to have low bioavailability in the presence of soy protein. There is some evidence that iron, copper and manganese in soy protein are less well utilized than when consumed in animal proteins. Soy protein and soy products generally contain 1.0-1.5% phytate, a compound that is clearly implicated in decreasing the bioavailability of zinc. The detrimental effect of phytate is aggravated by excess calcium. Under some conditions phytate decreases iron bioavailability, but iron absorption from soy protein generally appears to approach that of animal proteins. Fiber has been implicated in decreasing zinc absorption, but the low concentration of crude fiber in soybean products in general and in soy protein in particular suggests that fiber is responsible for little of the low zinc availability associated with these products. With the proper processing of soy protein so as to lower phytate, the nutritional value of soy protein could be substantially improved.

REFERENCES

Anderson, G. H.; Harris, L.; Rao, A. V. and Jones, J. D. (1976) *J. Nutrition 106*, 1166-1174.

Bassiri, A. and Nahapetian, A. (1977) *J. Agr. Food Chem. 25*, 1118-1122.

Boland, A. R.; Garner, G. B. and O'Dell, B. L. (1975) *Agr. Food Chem. 23*, 1186-1189.

Bowering, J.; Sanchez, A. M. and Irwin, M. I. (1976) *J. Nutrition 106*, 987-1074.

Byrd, C. A. and Matrone, G. (1965) *Proc. Soc. Exp. Biol. Med. 119*, 347-349.

Cartter, J. L. and Hopper, T. H. (1942) *USDA Tech. Bull.* 787.

Cowan, J. W.; Esfahani, M.; Salji, J. P. and Azzam, S. A. (1966) *J. Nutrition 90*, 423-427.

Davies, N. T. and Nightingale (1975) *Brit. J. Nutrition 34*, 243-258.

Davis, P. N.; Norris, L. C. and Kratzer, F. H. (1962) *J. Nutrition 77*, 217-223.

Ellis, R. and Morris, E. R. (1978) *Fed. Proc. 37*, 585 (Abst.).

Erdman, J. W.; Weingartner, K. E.; Parker, H. M. and Forbes, R. M. (1978) *Fed. Proc. 37*, 891 (Abst.).

Evans, G. W. and Johnson, P. E. (1977) *Fed. Proc. 36*, 1100 (Abst.).

Ferretti, R. J. and Levander, O. A. (1976) *J. Agr. Food Chem. 24*, 54-56.

Fitch, C. D.; Harville, W. E.; Dinning, J. S. and Porter, F. S. (1964) *Proc. Soc. Exp. Biol. Med. 116*, 130-133.

Ford, J. R.; Mustakas, G. C. and Schmutz, R. D. (1978) *J. Amer. Oil Chemists Soc. 55*, 371-374.

Forbes, R. M. (1961) *J. Nutrition 74*, 194-200.

Forbes, R. M. (1964) *J. Nutrition 83*, 225-233.

Forbes, R. M. and Parker, H. M. (1977) *Nutrition Reports International 15*, 681-688.

Fritz, J C.; Pla, G. W.; Roberts, T.; Boehne, J. W. and Hove, E. L. (1970) *J. Agri. Food Chem. 18*, 647-651.

Fuhr, I. and Steenbock, H. (1943) *J. Biol. Chem. 147*, 59-64.

Green, J. D.; McCall, J. T.; Speer, V. C. and Hays, V. W. (1962) *J. Animal Science 21*, 997 (Abst.).

Greger, J. L.; Abernathy, R. P. and Bennett, O. A. (1978) *Am. J. Clin. Nutrition 31*, 112-116.

Guthrie, B. E. and Robinson, M. F. (1978) *Fed. Proc. 37*, 254 (Abst.).

Imail-Beigi, F.; Reinhold, J. C.; Faradji, B. and Abadi, P. (1977) *J. Nutrition 107*, 510-518.

Kratzer, F. H.; Allred, J. B.; Davis, P. N.; Marshall, B. J. and Vohra, P. (1959) *J. Nutrition 68*, 313-322.

Lease, J. G.; Barnett, B. D.; Lease, E. J. and Turk, D. E. (1960) *J. Nutriton 72*, 66-70.

Likuski, H. J. A. and Forbes, R. M. (1964) *J. Nutrition 84*, 145-148.

Likuski, H. J. A. and Forbes, R. M. (1965) *J. Nutrition 85*, 230-234.

Lo, G. S. (1978) Personal Communication from Ralston Purina Co.

Lo, G. S.; Collins, D. W.; Steinke, F. H. and Hopkins, D. T. (1978) *Fed. Proc. 37*, 667 (Abst.).

Lolas, G. M. and Markakis, P. (1975) *Agr. Food Chem. 23*, 13-15.

Lolas, G. M.; Palamidis, N. and Markakis, P. (1976) *Cereal Chem. 53*, 867-871.

McCance, R. A.; Edgecombe, C. N. and Widdowson, E. M. (1943) *Lancet 2*, 126-128.

Meyer, E. W. (1978) Personal Communication. Central Soya Co.

Morris, E. R. and Ellis, R. (1976) *J. Nutrition 106*, 753-760.

Morris, E. R. and Ellis, R. (1978) *Proc. 10th National Conference on Wheat Utilization* (In press) also personal communication.

Momcilovic, B.; Belonje, B.; Giroux, A. and Shah, B. G. (1976) *J. Nutrition 106*, 913-917.

Oberleas, D.; Muhrer, M. E. and O'Dell, B. L. (1962) *J. Animal Science 21*, 57-61.

Oberleas, D.; Muhrer, M. E. and O'Dell, B. L. (1966) *J. Nutrition 90*, 56-62.

O'Dell, B. L. (1969) *Amer. J. Clinical Nutrition 22*, 1315-1322.

O'Dell, B. L. (1972) *Annals N. Y. Acad. Sciences 199*, 70-81.

O'Dell, B. L. and Savage, J. E. (1957) *Fed. Proc. 16*, 394 (Abst.)

O'Dell, B. L. and Savage, J. E. (1960) *Proc. Soc. Exp. Biol. Med. 103*, 304-306.

O'Dell, B. L.; Yohe, J. M. and Savage, J. E. (1964) *Poultry Science 43*, 415-419.

O'Dell, B. L.; Burpo, C. E. and Savage, J. E. (1972a) *J. Nutrition 102*, 653-660.

O'Dell, B. L.; Boland, A. R. and Koirtyohann, S. R. (1972b) *J. Agr. Food Chem. 20*, 718-721.

O'Dell, B. L. and Boland, A. R. (1976) *Agr. Food Chemistry 24*, 804-808.

Osborn, T. W. (1977) *J. Agr. Food Chem. 25*, 229-232.

Ranhotra, G. S.; Loewe, R. J. and Pergot, L. V. (1974) *Cereal Chemistry 51*, 323-329.

Ranhotra, G. S.; Loewe, R. J. and Puyat, L. V. (1974) *J. Food Science 29*, 1023-1025.

Reinhold, J. C.; Faradji, B.; Abadi, P. and Ismail-Beigi, F. (1976) *J. Nutrition 106*, 493-503.

Reinhold, J. G.; Nasr, K.; Lahimgarzadeh, A. and Heydayati, H. (1973) *Lancet 1*, 283-288.

Reinhold, J. G.; Parsa, A.; Karimian, N.; Hammick, J. W. and Ismail-Beigi, F. (1974) *J. Nutrition 105*, 976-982.

Sandstead, H.; Klevay, L.; Munoz, J.; Jacob, R.; Logan, G.; Dintzis, F.; Inglett, G. and Shuey, W. (1978) *Fed. Proc. 37*, 254 (Abst.).

Savage, J. E.; Yohe, J. M.; Pickett, E. E. and O'Dell, B. L. (1964) *Poultry Science 43*, 420-426.

Sharpe, L. M.; Peacock, W. C.; Cook, R. and Harris, R. S. (1950) *J. Nutrition 41*, 433-446.

Smith, W. H.; Plumlee, M. P. and Beeson, W. M. (1962) *J. Animal Science 21*, 399-405.

Smith, A. K. and Rackis, J. J. (1957) *J. Amer. Chem. Soc. 79*, 633-637.

Steinke, F. H. and Hopkins, D. T. (1978) *J. Nutrition 108*, 481-489.

Welch, R. M.; House, W. A. and Allaway, W. H. (1974) *J. Nutrition 104*, 733-740.

Welch, R. M. and van Campen, D. R. (1975) *J. Nutrition 105*, 253-256.

DISCUSSION

Erdman: I agree with your overall conclusion that zinc is

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Ismail-Beigi,
. and Heydayati,
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76-982.
, R.; Logan, G.;
ed. Proc. 37,
and O'Dell,
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er. Chem. Soc.
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H. (1974) J.
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on that zinc is

the mineral of most concern when considering the availability of minerals from high phytate foods. At the University of Illinois in conjunction with Dr. R. M. Forbes we have been conducting extensive studies concerned with the bioavailability of zinc, iron, calcium and magnesium from soybean foods using slope ratio assay procedures.

There are several issues that concern phytate and mineral availability that should be mentioned at this point. First of all in considering soy protein products, we must be concerned with the bioavailability of minerals from the soy product itself as well as the effects of the presence of soy protein upon the bioavailability of minerals from the rest of the diet. Thus far in our studies we have found that the presence of soy products in animal diets have little or no effect upon the bioavailability of inorganic salts added to these diets. This would suggest that extending chopped meat with soy protein for example should not effect the iron bioavailability from the meat.

We will present work later this year demonstrating that the inclusion of soybean hull, which is about 50% fiber, into soy-flour based diets has no effect upon the bioavailability of zinc and calcium for the rat.

Finally, I would like to bring up the area of food processing and the formation of insoluble phytic acid - mineral and phytic acid - mineral - protein complexes. Knowledge of solubility characteristics of protein, phytic acid and various phytate salts in various pH regions during processing of soy protein products may aid in predicting the extent of mineral binding and, therefore, mineral bioavailability. Perhaps Joe Rackis would like to speak to this issue, as he has been working in this area for several years.

O'Dell: I wish to emphasize the importance of the phytate to zinc ratio in considering the effect of phytate on zinc availability. If the molar ratio becomes greater than 20, there is likely to be decreased absorption of zinc regardless of the source of zinc in the diet.

Rackis: I wish to emphasize that reports suggesting soy protein inhibits the body's ability to utilize zinc has created great controversy. Part of the problem is the failure of the investigators to make a distinction between flours, concentrates, and isolates and to describe conditions of preparation of the products. Not all problems with mineral bioavailability are associated with phytic acid content *per se*. A case in point, zinc availability in the isoelectric form of soy protein isolate, specifically manufactured for use in infant formulas, is high, while some isolates modified by alkali and other conditions of processing exhibit low bioavailability. Yet, both types have similar amounts of phytic acid. Since such a variety of soy protein products are available, I would suggest that as much information as possible concerning processing history should become a part of all investigations on the nutritional quality of soy products.

O'Dell: I heartily agree that not all problems of mineral bioavailability in foods are related to their phytate content. However, it is clear that the addition to diets of soluble phytate, either the sodium salt or the acid, decreases the availability of zinc. This is the case when it is added to either casein or amino acid based diets. Thus, it is clear that a protein-phytate complex is not essential for zinc binding. It is possible, as we postulated early in our investigations, that some protein-phytate-zinc complexes make zinc less readily absorbed than others. So far as I know, there is no good evidence to support this concept.

Bodwell: For the most part, the few studies such as those of Reinhold which have attempted to clarify the relation between dietary phytate and mineral retention in humans have been confounded by the presence of fiber. A study with primates was done several years ago by Fitch and others in which an undefined source of soy protein was shown to cause severe anemia. Supplementation with iron clearly reversed the effect. This suggests that there may be deleterious effects of dietary phytate on mineral retention in primates including humans. If this is so, it would probably be a serious problem only with the extensive use of meat analogs. This may occur and it is pertinent to suggest that we seriously need some definitive studies with humans to explore the phytate-mineral retention issue. Would you care to comment?

O'Dell: I agree that we need human studies.

Liener: Do you feel that soy protein has a rachitogenic effect?

O'Dell: Dr. Liener's question refers to the observations of Carlson et al, made about 1964, that an isolated soy protein has a rachitogenic effect in turkey poults. The effect was largely overcome by high levels of Vitamin D. Autoclaving the protein did not prevent its rachitogenic effect. It appeared that the protein interfered with absorption of calcium and/or phosphorus. An explanation for the effect is not known, but it is conceivable that a soy protein-phytate complex was involved.

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Erdman: The calcium content of soy protein products is quite low. For example, a rat fed a 20% soy protein diet from full fat soy flour will recieve only 10% of its calcium requirement from the soy. Soy based diets must be fortified with an additional calcium source. We have studied the bioavailability of calcium added as calcium carbonate to soy flour, soy beverage and soy concentrate based diets and have found full bioavailability of added calcium. It is, therefore, safe to assume that rickets development in experimental animals must have been caused by some other factor such as lack of Vitamin D or poorly available phosphorus.