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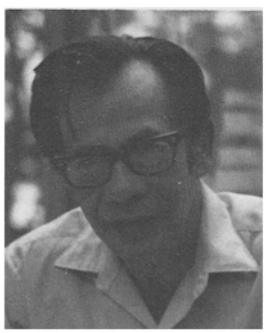

Factors Affecting the Nutritional Quality of Soya Products

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ABSTRACT

The nutritional quality of soya products is determined not only by the quantity and availability of the amino acids which make up the protein of such products, but is also markedly affected by the processing conditions which are employed in their manufacture. The most important factor in this regard is the application of some form of heat treatment which serves to inactivate a number of naturally occurring constituents of the soybean. Although non-toxic in the truest sense of the word, these are substances which can nevertheless elicit adverse physiological responses in animals, and, unless destroyed, can detract from the full nutritional potential of soya protein. The best known and certainly the most studied of these factors are the inhibitors of trypsin and chymotrypsin, enzymes which play a key role in the digestion of proteins in animals. These will be discussed with respect to their possible model of action *in vivo*, and an attempt will be made to evaluate their nutritional significance in man. Also present in soybeans are several other heat-labile components whose physiological significance is less well understood. These include the phytohemagglutinins (lectins), goitrogens, antivitamins and phytates. Less sensitive to the destruction effects of heat are a number of factors which are capable of producing a wide variety of physiological responses in animals and include saponins, estrogens, oligosaccharides and allergens. Although present in readily detectable quantities, their effect on the nutritional quality of soya protein is questionable. Not to be overlooked is the fact that harsh processing conditions such as excessive heat treatment or extraction under alkaline conditions may lead to the destruction of amino acids or to the formation of lysinoalamine. Although of lesser economic importance at the present time, brief consideration will be given to the possible effects of germination and fermentation on the nutritive quality of the protein resulting from such treatments.

INTRODUCTION

Ever since the soybean was introduced into the U.S., it was clearly recognized as an extremely valuable source of protein not only for feeding animals but, more recently, in the diet of man, as well. Although the high nutritional value of the soybean is determined largely by the amino acid composition of the protein, its full nutritional potential is attained only after a certain amount of heat treatment has been applied. Implicit in this observation is the realization that there are heat-labile factors present in soybeans which can interfere with utilization of its protein. In addition to those factors that are inactivated by heat, other factors are known to be present which are not destroyed by heat and which can also detract from the nutritional quality of soybean protein, albeit to a relatively minor extent and only under rather special circumstances. Table I is a compilation of the heat-labile and heat-stable antinutritional factors known to be present in soybeans, although, in some cases, only partial inactivation by heat may occur so that strict assignment to one or other of these two categories may be somewhat arbitrary. Each of these factors will be discussed in turn, and an attempt will be made to evaluate their nutritional significance, particularly in the human diet, and how their effects may be eliminated by appropriate processing methods.

TABLE I

Antinutritional Factors in Soybeans

Heat-labile	Heat-stable
Trypsin inhibitors	Saponins
Hemagglutinins	Estrogens
Goitrogens	Flatulence factors
Antivitamins	Lysinoalanine
Phytates	Allergens

HEAT-LABILE FACTORS

Protease Inhibitors

The protease inhibitors (more commonly referred to as trypsin inhibitors) are probably the best known, and certainly the most studied, of all of the antinutritional factors in soybeans. (Strictly speaking, the term protease inhibitor is preferred to trypsin inhibitor because this family of proteins inhibits a wide variety of proteinases in addition to trypsin. For comprehensive review of the chemical properties of soybean protease inhibitors, see Liener and Kakade [1]). It was not long after soybeans were introduced into the U.S., primarily as a source of oil, that Osborne and Mendel (2) made the significant observation that soybeans had to be heated in order to support the growth of rats. With the demonstration of a heat-labile trypsin inhibitor in soybeans and its subsequent crystallization (3), it was generally assumed that the beneficial effect of heat treatment could be ascribed to the destruction of this inhibitor. The inactivation of the trypsin inhibitor does, in fact, appear to parallel the improvement in nutritive value effected by heat as demonstrated with rats (Fig. 1). Further evidence came from experiments in which it was shown that the addition of purified preparations of the trypsin inhibitor to heated soybeans, to provide the same inhibitory activity as raw soybeans, caused a significant reduction in growth (Table II). It is important to note, however, that adding the trypsin inhibitor did not reduce the PER to the same level of growth as was observed on raw soybeans, indicating that heat treatment was doing something more than just inactivating the trypsin inhibitor. This is a point which will be discussed later.

With the recognition of the presence of a trypsin inhibitor in soybeans, it was tempting to conclude that the growth inhibition which it evoked in animals was simply due to an inhibition of digestion of dietary protein by proteolytic enzymes present in the intestinal tract. The most destructive blow to this theory was the observation that preparations of trypsin inhibitor were capable of inhibiting growth even when incorporated into diets containing predigested protein or free amino acids (5-7). Such experiments obviously rule out an inhibition of proteolysis as the sole factor responsible for growth inhibition, and thus served to focus attention on some alternative mode of action of the trypsin inhibitor.

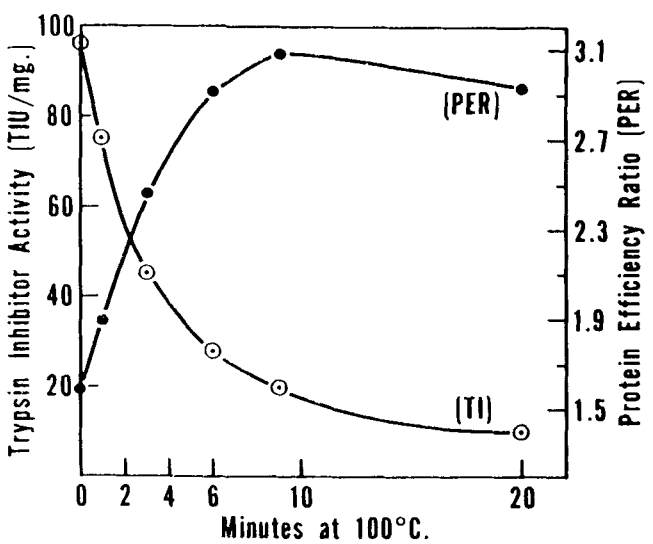


FIG. 1. Effect of heat treatment on trypsin inhibitory activity and protein efficiency ratio of soybean protein (4).

TABLE II

Effect of Adding Partially Purified Soybean Trypsin Inhibitor (STI) to Diets Containing Heated Soybean Meal in the Presence and Absence of Methionine (5)

Diet	PER - met	PER + met[a]
Raw soybeans	1.40	2.42
Heated soybeans[b]	2.63	2.99
Heated soybeans + 1.8% STI	1.95	2.63

[a]Diets were supplemented with 0.6% methionine.
[b]Autoclaved at 15 lb pressure (115 C) for 20 min.

Perhaps the most significant observation which has ultimately led to a better understanding of the mode of action of the soybean inhibitor was the finding that raw soybeans and the trypsin inhibitor itself could cause hypertrophy of the pancreas, an effect which is accompanied by an increase in the secretory activity of the pancreas (8). This led to the suggestion that the growth depression caused by the trypsin inhibitor might be the consequence of an endogenous loss of essential amino acids being secreted by a hyperactive pancreas (9,10). Because pancreatic enzymes such as trypsin and chymotrypsin are particularly rich in the sulfur-containing amino acids, pancreatic hypertrophy causes a drain on the body tissue of these particular amino acids in order to meet an increased need for the synthesis of these enzymes. This loss in sulfur-containing amino acids serves to accentuate an already critical situation with respect to soybean protein, which is inherently deficient in these amino acids.

The mechanism whereby the trypsin inhibitor induces pancreatic enlargement is still not fully understood. Green and Lyman (11), Schneeman and Lyman (12), and Lyman et al. (13) have shown that pancreatic secretion is controlled by a mechanism of feedback inhibition which depends upon the level of trypsin and chymotrypsin present at any given time in the small intestine. When the level of these enzymes falls below a certain critical threshold value, the pancreas is induced to produce more enzyme. The suppression of negative feedback inhibition can occur if the trypsin is complexed with the inhibitor or by dietary protein itself (see following). It is believed that the mediating agent between trypsin and the pancreas is the hormone cholecystokinin (CCK), which is released from the intestinal mucosa when the level of trypsin in the intestine falls below its threshold level. Those relationships are illustrated in Figure 2.

It was mentioned earlier that the trypsin inhibitor itself did not appear to account fully for the growth inhibition observed with raw soybeans (Table II). A further indication that this might be true came from an investigation of a large number of different varieties of soybeans in which the PER of such beans were compared with their trypsin inhibitor activity. As shown in Figure 3, there is no correlation whatsoever between these two parameters. Furthermore, if the trypsin inhibitor activity of a crude extract of soybeans is

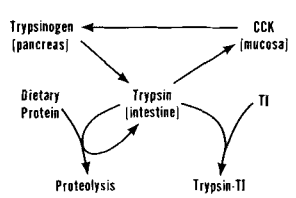


FIG. 2. Regulation of the secretion of trypsin by the pancreas. CCK, cholecystokinin; TI, trypsin inhibitor.

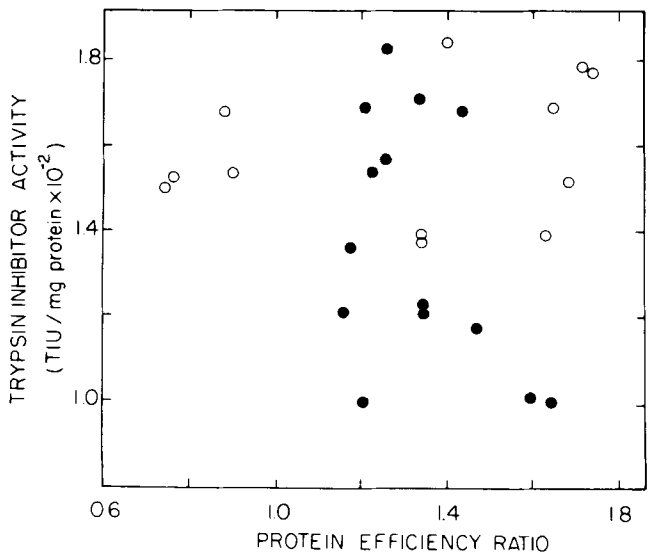


FIG. 3. Relationship of trypsin inhibitor activity to PER of different varieties of soybeans (14).

removed by affinity chromatography on Sepharose-bound trypsin, the resulting extract is still capable of causing growth inhibition and pancreatic hypertrophy (Table III). It may be estimated from these data that the trypsin inhibitor accounts for ca. 40% of the growth inhibition observed with raw soybeans. It is also significant to note that only ca. 40% of the enlargement of the pancreas produced by the ingestion of raw soybeans is also accounted for by the trypsin inhibitor.

These findings raise the question of what is responsible for the remaining 60% of the growth-retarding and pancreatic-inducing effects of raw soybeans. A clue comes from experiments in which the crude soybean extract from which the inhibitor had been removed was subjected to digestion with trypsin in vitro (Fig. 4). Heat treatment of this soybean protein produces an increase in the digestibility of the protein over and above the digestibility of a similar preparation from which the inhibitor had been removed. This observation suggests that native, undenatured soybean protein is in itself refractory to enzymatic attack unless denatured by heat. If this undenatured protein is, in fact, capable of binding trypsin by forming an enzyme-substrate complex as suggested by Green et al. (16), this undigested protein can likewise remove feedback inhibition of pancreatic secretion by trypsin. It would appear, therefore, that the trypsin inhibitor and the refractory nature of the soybean protein act through a common mechanism to inhibit the growth of rats.

It should be appreciated that all of the experiments described thus far were conducted with rats as the experimental model. As a basis for speculation as to the relevance of such experiments to humans, the following lines of evidence will be considered which suggest that the trypsin inhibitors are most likely of little consequence when soybean products are used for human food.

Many of the soybean products on the market today have been made from protein isolates which, depending on their mode of preparation, may contain as much as 30% of the trypsin inhibitor activity of the raw bean. An examination of the trypsin inhibitor activity of several textured meat analogs during the course of their manufacture reveals that, although the protein isolate may be rich in antitryptic activity, the inhibitor activity is reduced to very low levels in the final product (Table IV). Household cooking of such products would be expected to reduce these levels even further. Canned, frankfurter-type sausages containing 1.5% soya isolate were found to be essentially devoid of any trypsin inhibitor activity after the canning process (17). Furthermore, Nordal and Fossum (18) have reported that the trypsin inhibitor activity provided by soya isolate in meat products was actually more labile to heat inactivation due to some component in the meat ingredients. They postulated that this factor increased the sensitivity of the trypsin inhibitors to heat inactivation by causing the rupture of disulfide bonds in the inhibitor molecule, particularly the Bowman-Birk inhibitor, one of the trypsin inhibitors in soybeans which, unlike the Kunitz inhibitor, is rich in disulfide bonds.

Of particular concern to the pediatrician is the possi-

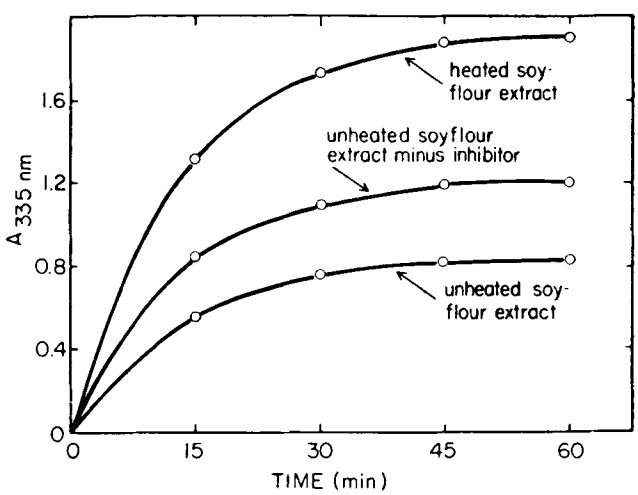


FIG. 4. In vitro digestibility by trypsin of soybean extract with and without trypsin inhibitor removed compared to the heated extract (15).

TABLE III

Contribution of Trypsin Inhibitors to Growth Inhibition and Pancreatic Hypertrophy Induced in Rats by Diets Containing Unheated Soybean Protein (15)

Source of protein	PER	Wt of pancreas (g/100 g body weight)
Soy flour extract, unheated	1.4	0.71
Soy flour extract, heated	2.7	0.57
Soy flour extract minus inhibitor[a]	1.9	0.65
Change due to removal of inhibitor (%)	+38	-41

[a]Trypsin inhibitors removed by passage of unheated soy flour extract through a column of Sepharose-trypsin.

TABLE IV

Trypsin Inhibitor Activities of Soybean Flour, Isolate, Fiber and Finished Textured Products[a]

	Antitrypsin activity (TIU[b] g dry solids x 10^{-3})	Soy flour (%)
Soy flour (unheated)	86.4	100
Soybean isolate	25.5	30
Soybean fiber	12.3	14
Chicken analog	6.9	8
Ham analog	10.2	12
Beef analog	6.5	7

[a]I.E. Liener, unpublished results.
[b]TIU = trypsin inhibitor units.

bility that infants fed soya milk formulated with isolate might be more sensitive to the same physiological effects of the inhibitor as observed in young rats. Churella et al. (19), however, have demonstrated that the heat treatment involved in the processing and sterilization of infant soya formulas containing soya isolate reduced the trypsin inhibitor activity to less than 10% of the original activity of the isolate. Mulne et al. (20) have also reported that most soya-based milk formulas have low but measurable levels of trypsin inhibitor activity. However, this activity apparently is too low to cause any weight reduction or pancreatic hypertrophy in rats (19). These observations are consistent with those of Rackis et al. (21), who found that no pancreatic hypertrophy occurred in rats fed soya flour in which only 54% of the trypsin inhibitor activity was destroyed, and maximal PER corresponded to a destruction of only 80% of the inhibitor activity of soya flour (Table V). In another series of experiments (22), it was found that soya products (commercial toasted soya flour, a protein concentrate and a protein isolate) which have residual activities ranging from 13 to 33% of the activity of raw soya flour produced normal pancreas when fed to rats for as long as 285 days.

Assuming, for the sake of argument, that the processing conditions may have been inadequate to reduce the level of trypsin inhibitory activity below the levels shown to be safe for rats, would the residual activity still pose a risk to human health? Let us first address the more basic question of whether the soybean inhibitors do, in fact, inhibit human trypsin. Trypsin inhibitor activity is invariably measured in vitro on the basis of the ability of soybean preparations to inhibit bovine or porcine trypsin because these are readily available commercially in "pure" crystalline form. Human trypsin is known to exist in two forms, a cationic species which constitutes the major component, and an anionic species which accounts for less than one-third of the total trypsin activity of pancreatic juice (23-25). As shown in Figure 5, while the minor anionic species of trypsin (trypsin 2) is inhibited by the soybean inhibitor in a stoichiometric fashion, the cationic species of trypsin (trypsin 1), which constitutes over two-thirds of the total trypsin activity of the human pancreas, is only very weakly inhibited. Incomplete inhibition of the trypsin activity of human duodenal aspirates (26) and of monkey pancreatic extracts (27) by soybean trypsin inhibitor have likewise been reported. On the other hand, the tryptic and chymotryptic activity of crude human pancreatic juice is completely inhibited by crude soybean extracts and purified inhibitors (28).

Another observation which serves to question whether the soybean protease inhibitors play any major role in human nutrition is the rather interesting relationship that seems to exist between the size of the pancreas of various species of animals and the nature of the response of their

TABLE V

Effect of Soy Flour Containing Various Levels of Trypsin Inhibitor on Growth and Size of Pancreas of Rats (21)

Trypsin inhibitor content (mg/100 g diet)	(% destruction)	Body wt (g)	PER	Pancreas wt (g/100 g body wt)
887	0	79	1.59	0.70
532	40	111	2.37	0.56
282	68	121	2.78	0.50
157	82	134	2.97	0.49
119	87	148	3.08	0.47
71	92	142	3.03	0.45
Casein	—	145	3.35	0.55

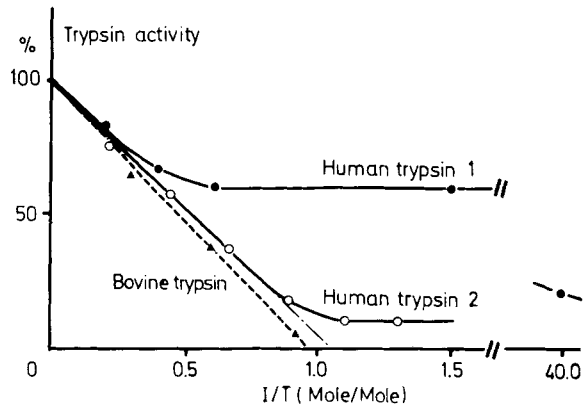


FIG. 5. Inhibition of human trypsins by soybean trypsin inhibitor. Trypsin 1, cationic species; trypsin 2, anionic species (24).

pancreas to the trypsin inhibitor. As shown in Table VI, there appears to be a direct relationship between the size of the pancreas and sensitivity of response to raw soybeans or the isolated inhibitor. Pancreases of those species of animals for which weights exceed 0.3% of the body weight become hypertrophic when fed raw soybeans or the inhibitor, whereas those animals whose pancreases fell below this value do not respond to the hypertrophic effects of the trypsin inhibitor. The guinea pig appears to be on the borderline of this relationship inasmuch as a positive response is noted in the case of the immature animal, but not in the case of the adult. One would predict from these data that the human pancreas would be insensitive to the effects of soybean inhibitor, although it must be emphasized that there is no direct experimental evidence bearing on this point.

Although live steam treatment (a process called toasting) is the most commonly used method of heat treatment for inactivating the protease inhibitors of soybeans, other forms of heat inactivation have proved effective for the inactivation of the inhibitors resulting in an improvement of the nutritive quality of soya protein. These include heating in boiling water (35) (such as might be used in home preparation of fresh green soybeans), dry roasting (36), dielectric heating (37), microwave radiation (38,39), micronization (40), and extrusion cooking (41). The trypsin inhibitor activity contributed by soybeans used in the preparation of Mexican tortilla is destroyed by treatment with 1% $Ca(OH)_2$ at 80 C for 1 hr (42).

The apparent improvement in the nutritive value of soybeans which accompanies germination (43-45) does not appear to be related to the trypsin inhibitor content be-

TABLE VI

Relationship between Size of Pancreas of Various Species of Animals and the Response of the Pancreas to Raw Soybeans or Trypsin Inhibitor

Species	Size of pancreas (% of body wt)	Pancreatic hypertrophy	Ref.
Mouse	0.6-0.8	+	29
Rat	0.5-0.6	+	1
Chick	0.4-0.6	+	1
Guinea pig	0.29	±[a]	30
Dog	0.21-0.24	-	31
Pig	0.10-0.12	-	32
Human	0.09-0.12	(-)[b]	33,34
Calf	0.06-0.08	-	

[a]Observed in young guinea pigs but not in adults.
[b]Predicted response.

TABLE VII

Biological Evaluation of a Soybean Line (PI-157440) Which Lacks the Kunitz Soybean Trypsin Inhibitor

Source of protein	Trypsin inhibitor activity[b]	Experiment with rats[c]		Experiment with chicks[d]	
		PER	Wt of pancreas (% of body wt)	Gain/feed	Wt of pancreas (% of body wt)
Raw soybeans[a]	100	0.98	0.61	0.40	0.25
PI-157440	40	1.44	0.54	0.45	0.19
Heated soybeans[a,e]	5	2.39	0.40	0.59	0.13

[a]Amsoy variety.
[b]Expressed as a percentage of activity in raw soybeans.
[c]Data of Tarcza and Liener (unpublished).
[d]Data of Bajjaleih et al. (59).
[e]Autoclaved at 15 lb pressure (120 C) for 15 min.

cause little if any change in trypsin inhibitor content seems to occur during germination (46-49). There is evidence, however, that modified forms of the Kunitz inhibitor appear during germination (50,51).

Soybean curd, or tofu, which is a popular dish in the Orient, is, in a sense, a protein isolate because it is the protein that is precipitated with calcium salt from a hot-water extract of the whole bean. The biological value of tofu is equivalent to that of properly processed soybean meal (52) or casein (53). Because the preparation of tofu involves the cooking or steaming of the beans prior to extraction with water, tofu is believed to be free of the trypsin inhibitor (54), although, surprisingly, no specific data on this point are available. Because fermented soybean preparations such as tempeh, natto and miso are generally made from boiled or autoclaved beans, they are virtually devoid of trypsin inhibitor activity (55). The slight increase in antitryptic activity which occurs as a consequence of fermentation (56,57) has been attributed to the release of free fatty acids from the oil by fungal lipase (56). This increase in antitryptic activity, however, is so slight that it does not affect weight gains of rats or the size of the pancreas (57).

Although considerable variation in trypsin inhibitor activity among different strains of soybeans has been observed (14), no strain has been found which is completely devoid of such activity. More recently, however, Orf and Hymowitz (58) discovered a soybean line from Korea which lacks the Kunitz inhibitor. Biological evaluation of this line in rats and chicks (Table VII) shows that it does, in fact, produce better growth response and lower pancreatic weights than a commercial variety of soybeans, although this improved response still falls short of heated soya flour. It is questionable, however, whether less heat treatment would be required to upgrade this particular line of soybeans as in the case with present commercial varieties of soybeans, because, as already pointed out, thermal denaturation of the protein is also necessary in order to achieve maximal digestibility of the protein.

Lectins

It has been recognized for many years that soybeans, in common with most other legumes, contain hemagglutinins (lectins) which have the unique property of binding carbohydrate substances (60). With red blood cells, the interaction of lectins with glycoproteins located on the surface of the cells is manifested in vitro by an agglutination of the cells. Ever since the days of Erhlich, it has been known that some of these lectins, such as ricin from the castor bean, are extremely toxic to animals. Soybeans contain several lectins comprising an estimated 1-3% of the protein of defatted soybean flour (61). There is, therefore, a definite possibility that these lectins might be responsible for the poor nutritive value of raw soybeans.

Soybean lectin, like the trypsin inhibitors, is readily destroyed by heat treatment, and destruction is accompanied by a marked improvement in the nutritive value of the protein (Fig. 6). When the isolated soybean hemagglutinin was fed to rats, the results obtained were somewhat ambiguous (63). As long as the animals were allowed free access to their food, there was a significant depression in

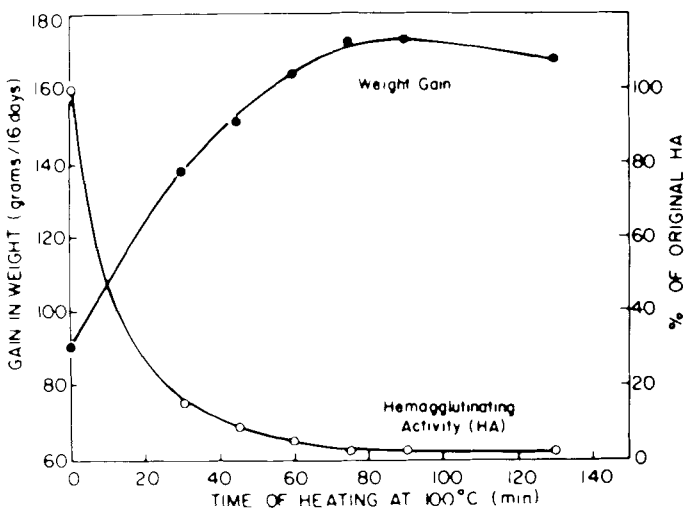


FIG. 6. Effect of heat treatment of soybeans on hemagglutinating activity and growth response of chicks (62).

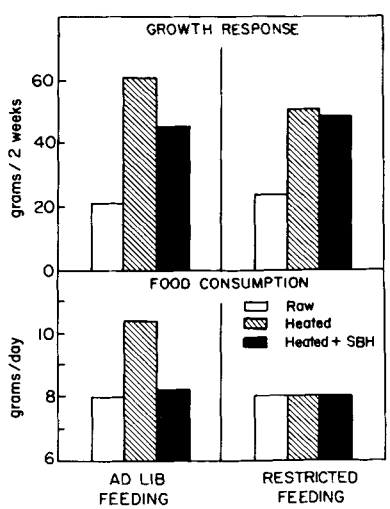


FIG. 7. Effect of adding specified soybean hemagglutinin to diet of rats under conditions of ad libitum and restricted feeding (63).

growth (Fig. 7). However, because this growth depression was accompanied by a concomitant decrease in food consumption, it was not clear whether the failure of the animals to grow was a consequence of lowered food intake or whether the lower food consumption was the result of depressed growth. When the food intake was equalized, however, the soybean hemagglutinin had little effect on growth. This negative effect was subsequently corroborated when it was found that rats fed soybean extracts from which the hemagglutinin had been removed selectively by affinity chromatography grew just as poorly as those receiving the original crude soybean extract (Table VIII). It would appear, therefore, that the soybean hemagglutinin, unlike those present in other beans (65), does not play any major role as a determinant of the nutritional quality of soybean protein.

Goitrogens

Unheated soybeans have been reported to cause marked enlargement of the thyroid gland of the rat and chick, an effect which could be counteracted by the administration of iodine (as KI) or partially eliminated by heat (66,67). A number of cases of goiter have also been reported in human infants fed soybean milk (68,69), a situation which could likewise be alleviated by iodine supplementation. An example of the therapeutic effectiveness of iodide in overcoming the goitrogenic effect of soyamilk fed to rats is shown in Figure 8. Iodine supplementation of soyamilk infant formulas is therefore recommended as a precautionary measure against the goitrogenic potential of this product (70).

The soybean component responsible for the goitrogenic effect of soybeans is still unknown. It appears to be concentrated mainly in the curd (72), although goitrogenic activity has also been detected to a lesser extent in toasted soya flours, concentrates and isolates (73,74). Unlike most goitrogenic plants belonging to the Cruciferae family, soybeans do not contain glucosinolates. The goitrogenic principle in soybeans has been reported to be a low molecular weight oligopeptide (75,76), which appears to be inconsistent with the finding that the goitrogenicity of soybean curd was not eliminated by proteolytic digestion (72). The bulk of the evidence would indicate that, whatever the goitrogenic principle is, it seems to exert its effect by inhibiting the uptake or incorporation of iodine into the thyroid gland. Other workers (69,77,78), however, believe that goiter may result from an increased fecal loss of thyroxine or a simple iodine deficiency (79,80). There has been one report that female rats which had been fed defatted soybeans with an iodine-free diet for 6-12 months showed thyroid carcinomas (81). This effect, however, was completely prevented by iodine supplementation.

TABLE VIII

Effect of Removing Soybean Hemagglutinin (SBH) on the Growth-Promoting Activity of Raw Soybean Extracts (64)

Protein component of diet	Hemagglutinating activity	PER
	Units/g protein x 10^{-3}	
Original soybean extract	324	0.91
Original soybean extract—SBH[a]	29	1.13
Original soybean extract, heated	6	2.25
Raw soy flour	330	1.01
Heated soy flour	13	2.30

[a]SBH was removed from an aqueous extract of soybeans by passage through a column of Sepharose-bound concanavalin A.

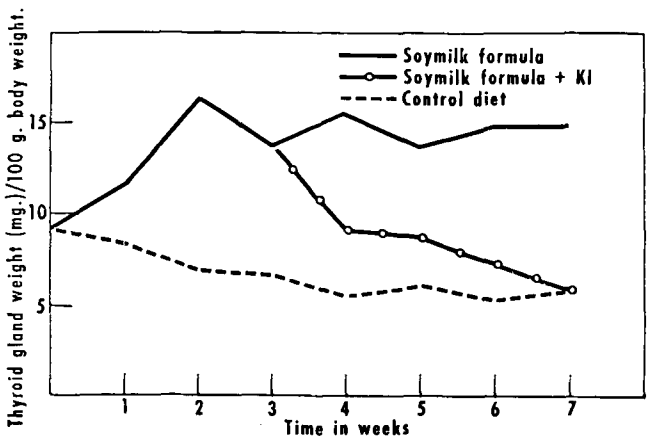


FIG. 8. Effect of soyamilk diet with and without iodide supplementation on the thyroid gland of the rat (71).

Antivitamins

Vitamin D. The inclusion of unheated soybean meal, or the protein isolated therefrom, in the diet of chicks may cause rickets unless higher than normal levels of vitamin D_3 are added to the diet (82). This rachitogenic effect can also be eliminated by autoclaving or by supplementation with calcium and phosphorus (83). It has been suggested that the rachitogenic properties of soya protein may be due to phytic acid (83), although the evidence on this point is not conclusive.

Vitamin E. Antivitamin E activity has been reported in isolated soya protein (84) as measured by growth, mortality, exudative diathesis and encephalomalacia. The identity of this antivitamin factor has not been established, although it has been suggested that it might be tocopherol oxidase (85).

Vitamin B_{12}. Not only is the soybean lacking in vitamin B_{12}, but it has also been reported to contain a heat-labile substance that increases the requirement for this vitamin (86) and causes an increased excretion of metabolites associated with enzymes that require vitamin B_{12} as a coenzyme (87). This increased requirement for vitamin B_{12} in rats fed raw soya flour has been attributed to a decreased availability of the vitamin produced by the intestinal flora and to an increased turnover of the absorbed vitamin.

Phytate

It is well recognized that the requirement for certain metals is increased in the presence of soybeans, an effect which has been attributed to its phytic acid content. Space does not permit a cursory, let alone a comprehensive, review of the numerous studies which have been conducted on the effect of soya protein on mineral availability. For a recent review of this subject, see a paper by O'Dell (88). Suffice to say that soybeans, and the various products derived therefrom, contain ca. 1- 1½ phytic acid which readily chelates with such di- and trivalent metal ions as calcium, magnesium, zinc, copper and iron. Such complexes are poorly absorbed from the intestines and thus results in reduced availability of these minerals from soybean products.

The reduced availability of zinc in such soya products as texturized vegetable protein (89) and soy-based infant formulas (90) is of particular concern because of the possibility of a marginal deficiency of zinc in the diets of some segments of the population. It would appear, however, from the studies at the University of Illinois (91) that, although zinc may be less available from soya flour itself, the soya flour does not interfere with the availability of zinc from other dietary sources or from mineral supplements. In

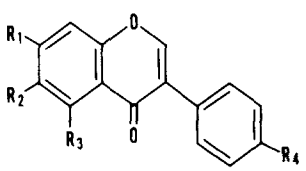
fact, experiments with human subjects extending over a period of two months in which one-fourth of the protein of a diet containing meat protein was replaced with soya protein showed very little disturbance in mineral metabolism, and what small changes were observed could be ascribed to changes in dietary composition (92).

Nevertheless, if soya proteins are to provide a major source of protein in the human diet, it would appear desirable to eliminate as much of the phytic acid as possible. Although this may be accomplished, at least in part, through the application of heat, other techniques which have been suggested include enzymatic hydrolysis, ion-exchange chromatography and close control of pH during the preparation of soya isolates (88).

HEAT-STABLE FACTORS

Estrogens

The banning of the use of diethylstilbesterol as a growth stimulant for animals in meat and poultry production has served to focus attention on the possible toxic effects of naturally occurring estrogens. In common with most plants, a number of compounds capable of eliciting an estrogenic response in experimental animals has been isolated from the soybean. These have been chemically categorized as isoflavones which exist in the plant as glycosides. Figure 9 shows the major isoflavones which have been isolated from soybeans, and their estrogenic potency relative to that of diethylstilbesterol is shown in Table IX. Also shown in this table is the related isoflavone coumestrol, which, although present in relatively low concentrations in soybeans and soybean protein preparations, is 70-150 times greater in germinated than in the ungerminated beans (96). These isoflavones in their isolated form can be demonstrated to interfere with the reproductive performance and to inhibit the growth of experimental animals when fed at sufficiently high levels (97-100). However, in order to attain such high



Genistein	R_1 = OH; R_2 = H; R_3 = OH; R_4 = OH
Genistin	R_1 = O-glucosyl; R_2 = H; R_3 = OH; R_4 = OH
Daidzein	R_1 = OH; R_2 = H; R_3 = H; R_4 = OH
Daidzin	R_1 = O-glucosyl; R_2 = H; R_3 = H; R_4 = OH
Glycitein	R_1 = OH; R_2 = OCH_3; R_3 = H; R_4 = OH
Glycitein-7-β-O-glucoside	R_1 = O-glucosyl; R_2 = OCH_3; R_3 = H; R_4 = OH

FIG. 9. Isoflavenoid compounds isolated from soybeans (93).

TABLE IX

Estrogenicity of Compounds Isolated from Soybeans

Estrogen	Concentration (ppm)	Relative potency (94)
Diethylstilbesterol[a]	–	1×10^5
Genistin	1644 (93)	1.00
Diadzin	581 (93)	0.75
Glycitein 7-O-β-glucoside	338 (93)	–
Coumestrol	0.4 (95)	35

[a]Included for comparative purposes.

levels from soybeans themselves, soybeans would have to be the sole constituent of the diet. It is unlikely, therefore, that the estrogens present in soybeans would constitute a health hazard to man as part of a normal, varied diet.

Saponins

Although saponins from some plants have an adverse effect on animal growth, it would appear that the saponins of soybeans are relatively innocuous to chicks, rats and mice, even when fed at levels three times greater than the levels found in soya flour (0.5%) (101). Saponins are hydrolyzed by bacterial enzymes in the lower intestinal tract, but neither saponins nor their aglycones (sapogenins) can be detected in the blood of test animals. It is probably safe to say that saponins should be removed from the list of anti-nutritional factors in soybeans.

Flatulence Factors

One of the important factors limiting the use of soybeans in the human diet is the flatulence associated with its consumption. The principle offenders appear to be low molecular weight oligosaccharides containing α-galactosidic and β-fructosidic linkages, namely raffinose and stachyose (Fig. 10). Thus, flatus activity in humans has been noted mostly with soybean products from which the carbohydrate has not been removed, such as full-fat and defatted soya flours (102,103). As shown in Table X, when soya flour is extracted with 80% ethanol to produce a concentrate, the flatulence effects are reduced considerably. Flatus activity resides mainly in the soya whey solids and in the alcohol extract which contain the low molecular weight oligosaccharides. Protein isolates, and products prepared therefrom, and fermented soy preparations such as tempeh (104) are virtually devoid of flatus activity.

Flatulence is generally attributed to the fact that man is not endowed with the enzyme (α-galactosidase) necessary for hydrolyzing the α-galactosidic linkages of raffinose and stachyose to yield readily absorbable sugars (105). Consequently, the intact oligosaccharides enter the lower intes-

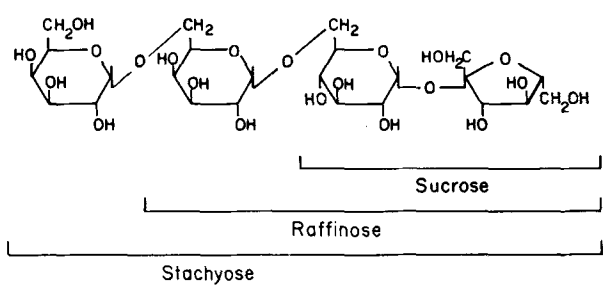


FIG. 10. Oligosaccharides believed to be responsible for flatulence-producing properties of soybeans.

TABLE X

Effect of Soy Products on Flatus in Man (102)

Soy product[a]	Flatus volume[b] (ml/hr)
Defatted flour	71
Protein concentrate	36
Whey solids	300
Alcohol extract	240
Protein isolate	13

[a]All soy products were toasted with live steam at 100 C for 40 min and fed at a level equivalent to 146 g defatted soy flour/day.
[b]Average of 4 subjects in each soy product.

tine where they are metabolized by the microflora producing such gases as carbon dioxide, hydrogen, and, to a lesser extent, methane. It is the production of these gases which are responsible for nausea, cramps, diarrhea and abdominal rumbling, and to the social discomfort generally associated with the ejection of rectal gases. It should be emphasized that there is considerable variability in individual response to the flatus-producing effects of beans. Many individuals are completely unaffected by the ingestion of beans, but the exact reason for this variable response is not understood completely, although it seems reasonable to assume that it is probably related to individual differences in the microbial population of the lower intestines.

Because the flatus-producing factors in soybeans are heat-stable, attempts have been made to eliminate these factors by enzymatic hydrolysis. Although treatment of soybeans with mold enzymes (106-109) or yeast fermentation (110,111) virtually eliminated stachyose and raffinose, there was no significant reduction in flatus activity in human subjects (104). Germination has also been reported to cause a marked reduction in the level of the offending oligosaccharides (112-115). Ultrafiltration of aqueous extracts of soybeans has been proposed as a means of removing oligosaccharides (116). Although there is a considerable variation in the raffinose and stachyose content of different varieties of soybeans (117), genetic changes by plant breeding is, at best, only a long-term solution to the problem.

Lysinoalanine

Alkaline extraction of soybeans, which is used frequently to prepare protein isolates, is known to reduce nutritive value of the protein, attributable, at least in part, to the destruction of cystine (118). One of the decomposition products of cystine is dehydroalanine (may also be derived from the decomposition of serine) which can interact with the ε-amino group of lysine to form lysinoalanine (Fig. 11). Alkali-treated soybeans have produced kidney lesions in rats, an effect which can be reproduced by the administration of free lysinoalanine (119). Inconsistent and variable results, however, have been reported by other workers (120-122), and it now appears that the response to lysinoalanine depends on the species of test animal (even among strains of the rat differences have been noted), composition of the basal diet, and whether the lysinoalanine is peptide-linked.

Sternberg et al. (123) have shown lysinoalanine to be widely distributed in cooked foods, commercial food preparations and food ingredients, many of which had never been subjected to alkaline treatment. Many of these foods had levels of lysinoalanine which were considerably higher than those found in commercial samples of soya protein isolate. Wide distribution of lysinoalanine among commonly cooked foods would tend to indicate that this is neither a novel or serious problem, because humans have long been exposed to proteins containing lysinoalanine with apparent impurity. Its presence in soya protein can hardly be considered a serious problem for man.

Aside from the question of the possible toxicity of lysinoalanine for man, the conversion of lysine to lysinoalanine may lead to a decrease in the digestibility of the protein (121) and a decrease in nutritionally available lysine (124). The latter effect may be of little nutritional consequence in the case of soybean protein which contains an excess of lysine, unless, of course, the soya protein is used to complement lysine-deficient cereal proteins.

Should the elimination of lysinoalanine prove to be a worthwhile and necessary objective, modification of existing processing methods should be considered which would minimize its formation. The feasibility of such an approach is indicated by Finley and Kohler (125) who found that the presence of reducing anions such as bisulfite, bisulfide and hypophosphite, and limited air incorporation significantly reduced the formation of lysinoalanine during alkaline processing. However, the possibility that the introduction of such chemicals may create other problems of toxicity should be carefully investigated.

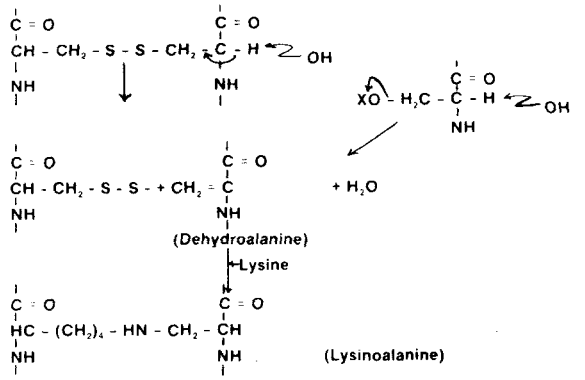


FIG. 11. Formation of lysinoalanine from cystine or serine and lysine.

Allergenicity

As the use of soybeans for food becomes more widespread, problems relating to soybean sensitivity or allergy might be anticipated. In contrast to antinutritional factors associated with foods, allergens display their effects only in those individuals possessing hypersensitivity to allergens; food allergens are generally innocuous when consumed by most people regardless of the amount ingested.

The immunochemical reactivity of most of the protein components of soybeans is destroyed by heat treatment (126-128), and this is reflected by the fact that heat-processed soybean products, including soyamilk, are generally considered to be hypoallergenic (129). Nevertheless, there may be found in the medical literature occasional case reports of adverse reactions of infants to soyamilk formula (130-134) and of children and adults who have ingested soybean products (135-138). The symptoms which have accompanied these reactions have all followed much the same pattern—nausea, diarrhea, vomiting and abdominal distress. The explanation for the allergenic response of some individuals to what would appear to be properly processed soybean products is not clear, but may be due to the fact that the heat required for the destruction of the allergenic principle is greater than that necessary for the inactivation of the major antinutritional factors in soybeans (127,129,139).

Identification of the specific soybean component responsible for the allergenicity of soybeans has proved to be elusive. In studies with animals, calves have proved to be particularly sensitive to even heat-treated soybean flours and exhibit severe gastrointestinal disturbance when placed on diets containing soybean meal (140-143). The histological characteristics of the intestinal mucosa of calves fed soybean meal are similar to those described in the acute response to soya protein seen in infants (140-142). It has been suggested that the soybean components most likely responsible for the allergic response in calves are glycinin and β-conglycinin, the major components of soya protein (143). These particular proteins were found to be relatively resistant to heat denaturation under the conditions employed for the manufacture of the soybean flour used in

these studies. It has been reported that extraction with hot aqueous alcohol removes or inactivates the allergenic factors (143,144); other workers (140-141), however, contend that the antigen may survive alcohol treatment. In studies with soya-sensitive children, the most potent allergen proved to be the 2S-globulin fraction of soybean protein which was likewise more heat-stable than the other protein fractions (136). The number of individuals who are sensitive to soybean protein must be rather low, because none of the 53 individuals who had a high dietary intake of soya protein in the form of texturized vegetable protein displayed any increase in circulating antibodies to soy protein (145). A recent paper (137) also implicated Kunitz soybean trypsin inhibitor as the factor being responsible for some cases of soybean allergy in humans.

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