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Effects of processing on antinutritional factors in legumes: The soybean case

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SUMMARY. The autor recounts his personal trail of research which has ultimately led to better understanding of the factors which contribute to the poor nutritive value of unheated soybeans. Among the 'techniques that were employed were the isolation of a lectin from raw soybeans, the use of affinity chromatography to remove the trypsin inhibitors, and the nutritional evaluation of soybean varieties which lacked the lectin or the Kunitz trypsin inhibitor. Based on a consideration of the results obtained by these experiments, it was estrimated that the trypsin inhibitors accounted for approximately 40% of the growth inihibiton on raw soy, of which two-thirds could be attributed to the Kunitz inhibitor and one-third to the Bowman-Birk inhibitor. The soybean agglutinin was deemed responsible for 50% of the inhibiton of growth, and the remaining 10% is most likely due to the poor digestibility of the undenatured protein.

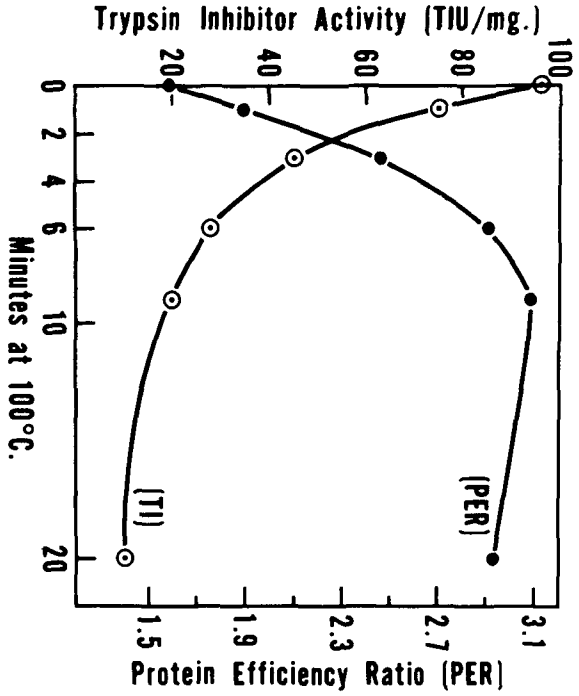
RESUMEN. Efecto del procesamiento sobre los factores antinutricionales de las semillas de leguminosa: el caso de la soya. El autor relata sus experiencias de investigación, que le han llevado finalmente a una mejor comprensión de los factores que contribuyen al pobre valor nutritivo de las semillas crudas de soya. Entre las técnicas empleadas figuran el asilameinto de la lectina de las semillas, el uso de cromatolografía de afinidad para remover los inhibidores de tripsina y la evaluación nutricional de variedades de soya desprovistas de lectina o del inhibidor de tripsina de Kunitz. En base al análisis de los resultados de dichos experimentos, se estimó que los inhibidores de tripsina contribuyen, aproximadamente, en un 40% a la inhibición del crecimento causado por la soya cruda; de este 40%, dos tercios pueden ser atribuidos al inhibidor de Kunitz y un tercio al de Bowman-Birk. La aglutinina de soya fue considerada como responsable del 50% de la inhibición del crecimiento. El restante 10% probablemente es debido a la pobre digestibilidad de las proteínas nativas.

BACKGROUND

My love affair with soybeans began more than four decades ago. Following my discharge from military service in 1946, I entered the PhD program in Biochemistry and Nutrition at the University of Southern California. After one year of intensive course work to get back on an academic track, I was made an offer I could not refuse. If I would agree to return to active duty, I would be assigned to the Quatermaster Corp in Chicago where, in addition to my military duties, I would be free to conduct my research for the PhD degree. One of the projects the Army was interested in at the time was the increased utilization of soybeans as a replacement for meat protein in army rations. One of the problems with the use of soybeans was the fact that, unless the soybean had been subjected to some from of processing, its nutritional value was very poor. I was asked to address this problem and to elucidate the basis for this phenomenon. Little did I realize at time that I was about to undertake a project which would become an almost life-time commitment.

Based on the early observation by Osborne and Mendel (1) it was already well known that the nutritive value of soybean protein poorly supported the growth of rats unless it had received some form of heat treatment. Also known at this time that soybeans in its raw state was a rich source of trypsin inhibitors (2). An example of the inverse relationship between the improvement in the nutritive value of soybean protein and the destruction of the trypsin inhibitor is shown in Fig. 1. This coupled with the observation that heat produced an improvement in the digestibility of the protein lead to what appeared to be the logical conclusion that the beneficial effect of heat treatment could be attributed to the destruction of these inhibitors that could otherwise interfere with the digestion of protein in the intestinal tract. Moreover, protein fractions which were highly enriched with trypsin inhibitor activity were capable of inhibiting the growth of rats, chicks, and mice (4-6).

FIGURE 1
Effect of heat treatment on the trypsin inhibitor activity and nutritive value of the protein as measured by the protein efficiency ratio (PER) rat assay. Taken from Rackis (3).



Despite these observatons it remained unclear why preparations of the trypsin inhibitor were capable of inhibiting growth even when incorporated into diets containing predigested protein or free amino acids (7). Such experiments obviously rule out an inhibiton of intestinal proteolysis as the sole factor responsabile for growth inihibition. Perhaps the most significant observaton that has ultimately led to better understanding of the mode of action of the soybean trypsin inhibitors was the finding that feeding of raw soybeans, or the purified inhibitors derived therefrom, caused an enlargement of the pancreas (8), which could be described histologically as hypertrophy as well as hyperplasia (9). Comcomitant with this increase in the size of the pancreas was an increase in the secretion of digestive enzymes, including trypsin, chymotrypsin, and elastase, and their ultimate excretion in the feces (10). Thus arose the hypothesis that the growth depression caused by the trypsin inhibitors was a consequence of an endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas. Because pancreatic enzymes such as trypsin and chymotrypsin are particulary rich in the sulfur-containing amino acids, the effect of a hyperactive pancreas is to divert these amino acids from the synthesis of body tissue protein to the synthesis of these enzymes which are subsequently lost in the feces. The net effect is a loss in weight because of an exacerbation of an already critical situation with respect to soybean protein, which is inherently deficient in the sulfur-containing amino acids.

But several observations cast doubt as to whether the destruction of the trypsin inhibitor by heat was in fact the whole answer to the problem. The addition of a concentrate of the trypsin inhibitor to diets containgn heated soybeans at a level equivalent to the trypsin inhibitor activity of raw soy did not reduce the PER to the same level as the raw soy (7). In other words there was a definite indication that heat treatment was doing something more than just destroying the trypsin inhibitor. Furhermore, in a study involving 26 varieties of soybeans, there was no correlation between the protein efficiency ratio (PER) as measured in rats and their trypsin inhibitor content (11). Interestingly enough, there was, however, a significant correlation between the PER and the size of the pancreas. This would indicate that some factor other than the trypsin inhibitors must also be playing a role stimulating the growth of the pancreas.

In order to delineate more precisely the role of the trypsin inhibitor, instead of adding the trypsin inhibitor to heated soybeans, we decided to remove only the trypsin inhibitor from unheated soybeans to see what effect this would have on the nutritive value of the protein. This approach would then exclude any effect heat treatment might have other than its effect on the trypsin inhibitor *per se*. When rats were fed a raw soybean extract from which trypsin inhibitor activity had been removed by affinity chromatography on Sepharose-trypsin, it was found that only apaproximately 40% of the difference in PER's and pancreas weights between raw and heated soybean protein could be atributed to the trypsin inhibitor (12).

We were thus faced with the question as to what might be responsible for the approximately 60% of the growth inhibition produced by the ingestion of raw soybeans by the rat. It was this consideration which prompted our search for the possible presence of a growth inhibitor (s) which might account for the failure to explain the beneficial effect of heat treatment as being due solely to the trypsin inhibitors. As a first approach to this problem we simply wanted to see if one could show the presence of a toxic factor by the direct intraperitoneal injection of a crude extract of raw soybeans into rats. The injection of the pure crystalline preparation of the Kunitz trypsin inhibitor proved innocuous, whereas the crude extract with considerably less antitryptic activity was in fact quite toxic (13). These results made it quite clear that there was something other than trypsin inhibitor activity that was producing a toxic response in rats. Thus began our search for some yet unidentified toxic factor in soybeans.

The most promising candidate appeared to be agglutinins known to be present in legumes. As early as 1888 Stillmark had already shown that ricin, the toxic principle of the castor bean, displayed hemagglutinating activity (14). Later, in 1908, Landsteiner and Raubitschek (15) had shown that crude extracts of many edible legumes, including soybean, also had hemagglutinating activity. Little attention, however, was paid to the possibiity that these agglutinins, which would later be referred to as «lectins», were responsible for the poor nutritive

value of some of these legumes in their raw form. It wasn't until latter part of '49 and the early '50's that Jaffé directed our attention to the possibility that the toxicity of raw beans (*Phaseoulus vulgaris*) might be due to the presence of these hemagglutinins (16-18). These reports prompted us to attempt the isolation of what we thought could very well be the toxic factor in soybeans that we were looking for. Using fractional precipitation of the protein with ammonium sulfate, we found that the intraperitoneal toxicity was closely associated with hemagglutinating activity but was completely unrelated to trypsin inhibitor activity (19). We managed to purify this toxic factor with its associated hemagglutinating activity to the point of homogeneity as evidenced by moving boundary electrophoresis and sedimentation in the ultracentrifuge. We subsequently deterimned some of its more important physicochemical parameters including its molecular weight (20), amino acid composition and end group analysis (21), and the effect of chemical modificatoin (22). These studies revealed that the soybean agglutinin was comprised of several polypeptide chains and was a glycoprotein, chemical features which subsequently proved to be characteristic of most other lectins.

As an aside it should be menitoned that at this time I took the liberty of naming this protein «soyin», a term which was intended to denote its relationship to the other toxic hemagglutinins that were known at the time, such as «rincin» derived from the castor bean (*Ricinus communis*) and «abrin» from the jequirity bean (*Abrus precatorius*). It was subsequently brought to my attention that the name «soyin» had been prevoiusly used to denote the proteolytic activity in a crude extract of the soybean (23), although the enzyme responsible for this activity was, to my knowledge, never isolated or characterized. Nevertheless, in deference to these investigators, and in order to avoid confusion in the literature, we no longer used the term «soyin» in subsequent papers from our laboratory. This protein has since been simply referred to as the soybean agglutinin or SBA. It may be of intrest to note that at the time that we reported the isolation of the soybean agglutinin in 1952 (19), the term «lectin» had not been introduced into the literature until two years later by Boyd and Shapleigh (24).

Up to this point we had succeeded in showing there was a hemagglutinin in soybeans which was toxic when injected. It remained to be proven, however, that this protein was in fact responsible, at least to some extent, for the poor nutritive value of raw soybeans when consumed in the diet. To prove this point it became necessary to develop a method for the large-scale preparation of SBA which would enable us to incorporate it into a diet fed to rats. A labor intensive procedure involving salt and alcohol fractionation enabled us to obtain from each kg of raw soy flour at least 2 gm of a hemagglutin preparation which we judged to be about 78% pure, based on electrophoretic analysis, and virtually devoid of antitryptic activity. This preparation was added to a diet containing heated soy flour at a level which would provide the same level of hemagglutinating activity as an equivalent level of raw soy flour. The inclusion of SBA into a diet containg autoclaved soy flour accounted for about half of the growth depression obtained with the raw flour (25).

More recently we were afforded the unique opportunity of having made available to us by Dr. T. Hymowitz of the U. Ill. a soybean strain which lacked the gene for the soybean lectin. The results of feeding rats this particular strain of soybeans, which had less than 0.05% of the activity of a commercial variety, fully confirmed our previous experiment in which we added SBA to heated soybeans, that is, the lectin-free soybean showed an improvement in nutritive value that was about half that produced by heating alone (26). See Table 1. Note that there is very little difference in nutritive value between these two cultivars following heat treatment. It is important to point out that the trypsin inhibitor content of both soybean varieities was essentially the same, thus ruling out any effect due to the trypsin inhibitors.

TABLE 1
NUTRITIONAL VALUE OF «LECTIN-FREE» SOYBEAN CULTIVAR (T102) COMPARED WITH CONVENTIONAL COMMERCIAL VARIETY OF SOYBEANS (AMSOY) AS MEASURED IN RATS[a]

Soybean	Weight gain (g/21 days)	PER[b]	Lectin activity (HU/mg protein)[c]
Raw Amsoy	9.1	0.64	314×10^3
Raw T102	18.2	1.21	120
Heated Amsoy	68.9	2.58	0
Heated T102	73.4	2.73	0

a Data taken from Donatucci (26).
b Protein Efficiency Ratio.
c HU = hemagglutinating units.

In more recent years research by Pusztai and his group as well as other workers have further investigated the antinutritonal effects of the soybean lectin. Among their findings was the surprising fact that the soybean lectin was also responsible for pancreatic enlargement (27). In addition the soybean lectin induced a low level of circulating insulin, an increased rate of lipid metabolism, and cellular hyperplasia of the small intestine (28,29). The latter effect could also lead to an increase in an endogenous loss of protein resulting in an inhibition of growth. Other adverse effects attributed to the soybean lectin include an inhibition of the disaccharidases and proteases in the intestines (30) and an interference with absorption of non-heme iron (31).

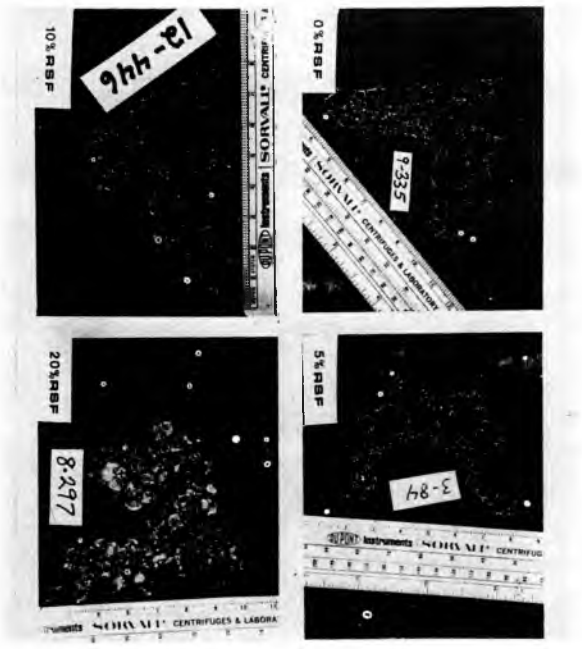
In addition to the trypsin inhibitors and lectin in soybeans another factor which must be taken into account is the digestibility of the protein itself. In order to dissociate the effect of the trypsin inhibitors on the digestibility of protein from the digestibility of the protein *per se, in vitro* digestibility studies were carried out on a crude extract of soybeans from which the trypsin inhibitors had been removed by affinity chromatography (12). A marked increase in the digestibility of

the soybean protein by trypsin was produced by heat treatment of the crude soybean extract. This increase, however, was much greater than the increase obtained with the unheated extract which was free of the inhibitor and may be attributed to the enhanced digestibility of the protein as a result of heat treatment. It should be mentioned that the resistance of native globulin proteins from the kidney bean to proteolytic attack had been reported prevoiusly from Jaffe's laboratory (32). It has been reported by Green *et al.* (33) that the presence of undigested protein in the small intestine can also cause an increase in pancreatic enzyme secretion. This it does in essentially the same way as the trypsin inhibitor, that is, by forming a stable enzyme-substrate complex with trypsin which results in removing the feedback inhibition of pancreatic secretion by trypsin.

Related to the increased proliferation of the pancreatic tissue evoked by the trypsin inhibitors is the finding that the long-term feeding of raw soyflour resulted in the production of adenomatous nodules on the pancreas (34). Prompted by these reports the USDA sponsered an in depth study in which rats were fed diets containinig various levels of raw soyflour so as to provide various levels of trypsin inihibitor activity (35). Fig. 2 compares the appearance of the pancreas of rats fed diets containing increasing levels of raw soy flour for a period of 18 months. A highly significant correlation was found between the incidence of nodules and the level of trypsin inhibitor activity in the diet.

FIGURE 2

Photograph of pancreas of rats fed diets containing increasing levels of raw soyflour (RSF) for a period of 18 months. Protein in diet was maintained at a constant level of 10% by appropriate mixtures of raw and heated soyflour. Taken from a study by Liener *et al* (35)



The effects of processing

Heat treatment. It is the relative ease with which the protease inhibitors and lectins are inactivated by moist heat treatment that has permitted the wide spread use of soybeans in animal and human diets. The general conclusion that can be drawn is that the extent to which the trypsin inhibitors and lectins are destroyed by heat treatment is a function of temperature, duration of heating, particle size, and moisture conditions. All of these factors are carefully monitored and controlled during the commercial production of soybean products in order to insure a product having maximum nutritional value. It is important to point out, however, that excessive heat treatment should be avoided in order to prevent damage to the nutritonal value of the protein. In seeking a compromise between these two apposing effects, it is not surprising that one generally finds small but measurable amounts of trypsin inhibitor activity in a variety of products containinig soy protein as the main source of protein (36).

Although these relatively low levels of trypsin inhibitor activity probably pose little risk to the general population, there are certain segments of the population that might be more vulnerable to the adverse effects of even low levels of the trypsin inhibitor. Since residual trypsin inhibitor activity may still remain in soy based infant formulas (37), infants who are fed soy milk for prolonged periods because of an allergy to cow's milk could be at risk. Another population group that might be at risk by continuous dietary exposure to the trypsin inhibitors are those individuals who are vegetarians, either by choice or culture, and most often choose legumes such as soybeans as a meat replacement. A similar situation would prevail in those individuals suffering from hyperlipidemia or hypercholesterolemia who have been advised to replace the animal protien with soybean protein in order to reduce the level of blood cholesterol.

Because of the compact structure of the Bowman-Birk inhibitor (BBI) and its stability towards heat in its purified state (38), it has been generally assumed that most of the residual trypsin inihibitor activity found in heat processed soybean products is due to this inhibitor. However, using a technique which serve to differentiate between the Kunitz inhibitor and BBI, it was the latter that was more readily destroyed than the Kunitz inhibitor (39). This difference in the heat resistance between the Kunitz inhibitor and BBI assumes added significance in view of reports of the anticarcinogenic properties of BBI (40). Thus, if one is prompted to preserve the BBI content of soybean products as a means of preventing cancer, more careful attention will have to be paid to the processing conditions used in producing such products.

The inactivation of the soybean lectin by moist heat treatment closely parallels the destruction of the trypsin inhibitors in soybeans. Since the soybean lectin is quite resistant to inactivation by dry heat treatment (41), this may explain why low but measurable levels of lectin activity were detected in a number of

soy containing products (42). It is doubtful, however, whether the final concentration of lectin in these products are such that they would pose a risk to human health.

Germination: Although the germination of soybeans has been reported to result in an improvement in the nuritive value of the protein, this effect appears to be unrelated to the level of trypsin inhibitor in the germinated bean (43). As far as the soybean lectin is concerned, germination is accompanied by a rapid disappearance of hemagglutinating activity (44). This perhaps may be one of the factors what accounts for the improved nutritional value of the germinated bean.

Traditional soybean dishes: Since the preparation of tofu, soymilk, and fermented dishes such as tempeh and natto generally involves the cooking or streaming of soybeans during or piror to extraction with water or fermentation, such dishes are generally quite low in trypsin inhibitor activity (45).

Chemical Treatment: Although heat is an effective and simple means of inactivating the trypsin inhibitors of soybeans, as already indicated, it carries with it the risk that excessive heat may damage the protein. Adjunct treatment with various thiol-containign chemicals, such as N-acetyl-cysteine and glutathione has been found to facilitate inactivation at lower temeratures (46). This inactivation is most likely a consequence of the interaction of the disulfide bonds of the trypsin inhibitors through the formation of mixed disulfides. The treatment of soy products with sodium sulfite, a reagent which is known to cleave disulfide bonds, likewise served to reduce the temperature necessary to inactivate the trypsin inhibitors (47).

Genetic Variants: Numerous studies have beeen devoted to a search for varieties of soybeans that might be low in trypsin inhibitor content, but, as already noted (11), there appears to be little correlation between trypsin inhibitor content and the nutritonal value of the protien. Hymowitz and coworkers have succeeded in identifying several isolines which lacked the Kunitz trypsin inhibitor but retained about 50% of the trypsin inhibitor activity of a common commercial variety of soybeans (48). This remaining activity was found to be entirely due to BBI. Feeding studies with several species of animals showed that the soybean isoline with reduced trypsin inhibitor activity supported better growth than raw soybeans containing the Kunitz inhibitor as well (48,49). An example of the performance of rats in terms of PER and the size of the pancreas when placed on a diet containing the soybean lacking the Kunitz inhibitor is shown in Table 2. From these data it may be calculated that approximately one-third of the growth inihibition and pancreatic enlargement produced by raw soy still remains after removal of the Kunitz inhibitor and may be presumed to be due to BBI. Heat treatment, however, still proved to be the most effective means for enchancing the nutritional value of the protein. The practical implication from these studies is the fact that milder heat treatment is needed to achieve near zero level of TI activity with the isoline laking the Kunitz inhibitor than with standard varieties of soybeans (49,51).

TABLE 2
BIOLOGICAL EVALUATION OF A SOYBEAN LINE (PI 147440) WHICH LACKS THE KUNITZ SOYBEAN INHIBITOR[a]

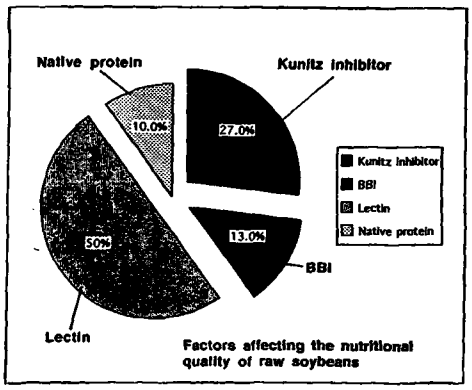
Soybean	TI activity[b]	PER[c]	Pancreas wgt.[d]
Unheated soy flour	100	0.98 (100)	0.61 (100)
Heated soyflour	5	2.39 (0)	0.40 (0)
Unheated PI 157440	40	1.44 (32)	0.54 (33)
Heated PI 157440	5	2.42 (102)	0.42 (90)

a Data taken from Tarcza (50).
b As percent of unheated soy flour.
c Protein efficiency ratio. Values in parentheses denote % of the growth inhibition obtained with unheated soy flour taken as 100%.
d Expressed as % of body weight. Values in parentheses denote % of the increase in weight of pancreas from unheated soy flour taken as 100%.

CONCLUSION

After decades of research the available evidence now permits an approximation as to how much of the beneficial effect of processing of soybeans is due to the inactivation of each of the antinutritional components shown in Fig. 3. The contribution of the trypsin inhibitors to the overall effect is about 40% of which two-thirds is due to the Kunitz inhibitor and one-third to BBI. The soybean lectin accounts for about 50% of the effect, and the remaining 10% must be due to the enhanced digestibility of denatured protein.

FIGURE 3
Pie chart showing the estimated contribution of the various antinutritional factors to the overall effect produced by raw soy beans. The inactivation of these factors by processing is responsible for the beneficial effect of heat treatment.



REFERENCES

1. Osborne T.B. & L.B. Mendel. The use of soybean as food. J. Biol. chem 32:369-387, 1917.
2. Kunitz M. Crystallization of a trypsin inhibitor from soybeans. Science 101:688-689, 1945.
3. Rackis J.J. Biological and physiological factors in soybeans. J. Amer. Oil Chem. soc. 51:161A-174A, 1974.
4. Klose A.A., B. Hill B. & H.L. Fevold. Presence of a growth inhibiting substance in raw soybean. Proc. Soc. Exp. Biol. Med. 62: 10-12, 1946.
5. Ham W.W., R.M. Sandstedt & F.E. Mussehl. The proteolytic inhibiting substance in the extract from unheated soybean meal and its effect on the growth in chicks. J Biol Chem 161:635-642, 1945.
6. Westfall R.J. & S.M. Hauge. The nutritive quality and trypsin inhibitor content of soybean flour heated at various temperatures. J. Nutr. 35:374-389, 1948.
7. Liener I.E.; H.J. Deuel Jr. & H.L. Fevold. The effect of supplemental methionine on the nturitive value of diets containining concentrates of the soybean trypsin inhibitor. J. Nutr. 39:325-339,1949.
8. Chernick S.S.; S. Lepkovsky & I.L. Chaikoff. A dietary factor regulating the enzyme content of the pancreas: changes induced in the size and the proteolytic activity of the chick pancreas by the ingestion of raw soybean meal. Amer. J. Physiol 155: 33-41, 1948.
9. Yanatori T. & T. Fujita. Hypertrohy and hyperplasia in the endocrine and exocrine pancreas of rats fed soybean trypsin inhibitor or repeatedly injected with pancreaozymen. Arch. Histol. Japan 39:67-68, 1976.
10. Nitsan Z. & I. E. Liener. Enzyme activities in the pancreas digestive tract, and feces of rats fed raw or heated soyflour. J. Nutr 106:300-305, 1976.
11. Kakade M.L.; N.R. Simons & I.E. Liener. Nutritonal and biochemical assessment of different varieties of soybean. J. Agric. Food Chem 20: 87-90, 1972.
12. Kakade M.L.; D.E. Hoffa & I.E. Liener. contribution of trypsin inhibitors to the deleterious effectos of unheated soybeans fed to rats. J. Nutr. 103:1772-1778, 1973.
13. Liener I.E. The intraperitoneal toxicity of concentrates of the soybean trypsin inhibitor. J. Biol. Chem 193:183-191, 1951.
14. Stilmark H. Uber Ricin. Arch. Phamakol. Inst. Dorpat. 3:57-62, 1889.
15. Landsteiner K. & H. Raubitschek. Beobachtungenuber Hamolyse und Hamagglutination. Zent. Bakteriol. Parasitenk. Abt/II, Orig. 45:660-667, 1908.
16. Jaffé W.G. Toxicity of raw kidney beans. Experientia 5: 81-83, 1949.
17. Jaffé W.G. La toxicidad de las caraotas crudas para conejos. Acta Cient. Venezolana 1:16-17, 1950.
18. Jaffé W.G. Estudio sobre la inhibición del crecimiento de ratas causada por algunas semillas de leguminosas. Acta Cient. Venezolana 1:62-64, 1950.
19. Liener I.E. & M.J. Pallansch. Purification of a toxic substance from defatted soy bean flour. J. Biol. Chem. 197:29-36, 1952.
20. Pallansch M.J. & I.E. Liener. Soyin a toxic protein from the soybean. II Physical characterization. Arch. Biochem Biophys. 145:366-374, 1953.
21. Wada S.; M.J. Pallansch & I.E. Liener. Chemical composition and end groups of the soybean agglutinin. J. Biol. Chem 233:395-400, 1958.
22. Liener I.E. & S. Wada. Chemical modification of soybean hemagglutinin. J Biol. Chem 222:695-704, 1956.
23. Laufer S.; H. Tauber & C.F. Davis. The amylolytic and proteolytic activity of soybean seed. Cereal Chem. 21:267-273, 1944.
24. Boyd W.C. & E. Shapleigh. Specific precipitating activity of plant agglutininis (lectins). Science 119:419, 1954.
25. Liener I.E. Soyin a toxic protein from the soybean. I. Inhibition of rat growth. J. Nutr 49: 527-539, 1953.
26. Donatucci D.A. The role of lectins in the nutritonal toxicity of raw legumes. PhD. Thesis, University of Minnesota.1983. p. 160-164.
27. Grant G.; W.B. Watt; J.C. Stewart & A. Pusztai. Effects of dietary soybean (*Glycine max*) lectin and trypsin inhibitors upon the pancreas of rats. IRCS Med. Sci. Res. 15:1197-1198, 1987.
28. Grant G.; W.B. Watt; C. Stewart & A. Pusztai. Metaboic and hormonal changes in rats resulting fromm consumption of kdney bean (*Pahesoulus vulgaris*) or soybean (*Glycine max*). Nutr. Rep. Int. 36: 763-772, 1987.
29. Grant G.; P.M. Dorward & A. Pusztai. Pancreatic enlargement is evident in rats fed diets containing raw soybeans (*Glycine max*) or cow peas (*Vigna unguiculata*) for 800 days but not in those diets based on kidney beans (*Phaseoulus vulgaris*) or lupinseed (*Lupinus angustifollius*). J. Nutr 123:2207-2215, 1993.
30. Jindal S.; G.L. Soni & R. Singh. Effects of feeding soybean lectin on intestinal digestive enzymes in albino rats. IRCS Med. Sci. Res 10:214, 1982.
31. Hisayasu S.; H. Orimo; S. Migita; Y. Ikeda; K. Satoh; S. Shinjo; S. Hirai & Y. Yoshino. Soybean protein isolate and soybean lectin inhibit iron absorption in rats. J. Nutr. 122:1190-1196, 1992.
32. Seidl D.S.; M. Jaffé & W.G. Jaffé. Digestibility and proteinase inhibitor action of kidney bean globulin. J. Agric. Food Chem 17:1318, 1969.
33. Green G.M.; B.A. Olds; G. Mathews & R. L. Lyman. Protein as regulator of pancreatic secretion in the rat. Proc. Soc. Exp. Biol. Med. 142:1162-1167, 1973.
34. Mcguiness E.e.; R.G.H. Morgan; D.A. Levison; D.Ñ.; Frape; D. Hopwood & K.G. Wormsley. The effects of long-term feeding of raw soy flour onthe rat pancreas. Scand J. Gastroenterol. 15:497-502, 1980.
35. Liener I.E.; Z. Nitsan; C. Sriangnam; J.J. Rackis & M.R. Gumbman. The USDA trypsin inhibitor study. II. Time related biochemical changes in the pancreas of the rat. Qual. Plant. Plant Foods Human Nutr. 35:243-257,1985.
36. DiPietro C.M. & I.E. Liner. Soybean protease inhibitors in foods. J. Food Sci. 54:606-609, 1989.
37. Brandon D.L.; A. H. Bates & M. Friedman. ELISA analysis of soybean trypsin inhibitors in processed foods. In: Nutritional and toxicological consequences of food processing M. Friedman (Ed.). New York, NY. Plenum Press p. 321-337, 1991.
38. Birk Y. Purification and properties of a highly active inhibitor of tyrpsin and chymotrypsin from soybeans. Biochim. Biophys. Acta 54:378-381,1961.

39. DiPietro C.M. & I.E. Liener. Heat inactivation of the Kunitz and Bowman-Birk protease inhibitors. J. Agric. Food Chem 37:39-44, 1989.
40. Yavelow J.; T.H. Finlay; A.R. Kennedy & W. Troll. Bowman-Birk soybean protease inhibitor as an anticarcinogen. Cancer Res. 43: 2454s-2459s, 1983.
41. de Muelenaere H.J.H. Effect of heat treatment on the hemagglutinating activity of legumes. Nature 201:1029-1030, 1964.
42. Calderón de la Barca A.M.; L. Vásquez-Moreno & M.R. Robles-Burgueno. Active soybean lectin in foods: isolation and quantification. Food Chem 39:321-327,1991.
43. Desikachar H.S.R. & S.S. De. Role of inhibitors in soybean. Science 106:421-422,1947.
44. Chen L.H.; R.R. Thacker & S.H. Pan. Effect of germination on hemagglutinating activity of pea and bean seeds. J. Food Sci. 42:1666-1667,1977.
45. Hackler L.R.; J.P. van Bruen; K.H. Steinkrause; E.E. Rawi & D.B. Hand. Effect of heat treatment on nutritive value of soy milk protein fed to weanling rats. J. Food Sci. 30:723-728,1965.
46. Friedman M.; O.K.K. Grosjean & J.C. Zahnley. Inactivation of soya bean trysin inhibitors by thilos. J. Sci. Food Agric. 33: 165-172,1982.
47. Friedman M. & M.R. Gumbmann. Nutritional improvement of soy flour through inactivation of trypsin inhibitors by sodium sulfite. J. Food Sci. 51:1239-1241,1986.
48. Hymowitz T. Genetics and breeding of soybeans lacking the Kunitz tyrypsin inhibitor. In: Nutritional and toxicological significance of enzyme in hibitors in Foods. M. Friedman (Ed.) New York. N.Y. Plenum Press. p. 291-298, 1986.
49. Friedman M.; D.L. Brandon; A.H. Bates & T. Hymowitz. Comparison of commercial and an isoline lacking the Kunitz trypsin inhibitor: composition, nutritional value, and effects of heating. J. Agric. Food Chem 39:327-335,1991.
50. Tarcza J.C. and I.E. Liener. Unpublished data.
51. Liener I.E. & S. Tomlinson. Heat inactivation of soybean line lacking the Kunitz trypsin inhibitor. J. Food Sci. 46:1354-1356,1981.