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Nutritional Significance of Lectins and Enzyme Inhibitors from Legumes

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Legumes have natural components, such as lectins, amylase, and trypsin inhibitors, that may adversely affect their nutritional properties. Much information has already been obtained on their antinutritional significance and how to inactivate them by proper processing. Chronic ingestion of residual levels is unlikely to pose risks to human health. On the other hand, the ability of these molecules to inhibit some enzymes such as trypsin, chymotrypsin, disaccharidases, and α-amylases, to selectively bind to glycoconjugates, and to enter the circulatory system may be a useful tool in nutrition and pharmacology. Trypsin inhibitors have also been studied as cancer risk reducing factors. These components seem to act as plant defense substances. However, increased contents may represent an impairment of the nutritional quality of legumes because these glycoproteins and the sulfur-rich protease inhibitors have been shown to be poorly digested and to participate in chemical reactions during processing reducing protein digestibility, a still unsolved question.

KEYWORDS: Legumes; lectins; enzyme inhibitors

INTRODUCTION

Protein inhibitors of hydrolases are found in plants, animals, and microorganisms and are active against proteases, amylases, lipases, glycosidases, and phosphatases, those inhibiting proteases being the most well-known. From the nutritional aspect, the inhibitors of the serine proteases trypsin and chymotrypsin found in plant foodstuffs are the most important (*1*). Among them, trypsin inhibitors in soybeans and soy products have been the most extensively studied due to their importance in human and animal nutrition, although lectins, trypsin, and α-amylase inhibitors in common beans are also well characterized. Indeed, beans are the second largest group of seeds after cereals reported as natural sources of α-amylase inhibitors (*2*). Protease inhibitors in soybeans contain no carbohydrates and belong to two different families, referred to as Kunitz and Bowman−Birk. Kunitz type inhibitors have a molecular mass of ∼20 kDa, with two disulfide bridges, and act specifically against trypsin. Bowman−Birk type inhibitors have a molecular mass in the range of 8−10 kDa, with seven disulfide bridges, and inhibit trypsin and chymotrypsin simultaneously at independent binding sites. In common beans, lima beans, cowpeas, and lentils protease inhibitors have been characterized as members of the Bowman−Birk family (*1*, *3*). Lectins are glycoproteins also widely distributed in nature, including plants consumed as part of the human diet, and have the ability to combine reversibly and specifically with sugars and glycoconjugates. As a result, in addition to erythrocyte agglutination, they have numerous physiological effects, such as binding to glycoproteins on the epithelial surface of the small intestine, interfering with nutrient absorption. Kidney bean phytohemagglutinin (PHA) is a tetrameric glycoprotein consisting of two different subunits with a molecular mass of ∼120 kDa (*4*).

ENZYME INHIBITORS AND LECTIN CONTENTS IN LEGUMES

Grant et al. (*5*) determined trypsin inhibitor and lectin contents of kidney beans (*Phaseolus vulgaris*), soybeans (*Glycine max*), cowpeas (*Vigna unguiculata*), and lupin seeds (*Lupinus augustifolius*). They reported high levels of lectins in kidney beans [$(840 \pm 300) \times 10^{-5}$ hemagglutinating activity units (HU)/kg], moderate levels in soybeans [$(50 \pm 10) \times 10^{-5}$ HU/kg], and very low amounts in cowpea and lupin seeds [$(3 \pm 1) \times 10^{-5}$ HU/kg]. Protease inhibitor content was higher in soybeans (24.6 ± 1.5 g of trypsin inhibited/kg and 12.0 ± 1.8 g of chymotrypsin inhibited/kg), moderate in kidney beans and cowpeas (8.0 ± 0.8 g of trypsin inhibited/kg and 9.2 ± 0.9 g of chymotrypsin inhibited/kg; and 10.6 ± 1.5 g of trypsin inhibited/kg and 9.2 ± 1.1 g of chymotrypsin inhibited/kg, respectively), and low in lupin seeds (1.1 ± 0.8 g of trypsin inhibited/kg and 1.4 ± 0.8 g of chymotrypsin inhibited/kg). Pustzai et al. (*6*) reported a lectin content of 0.22%, a Kunitz inhibitor content of 0.45%, and a Bowman−Birk inhibitor content of at least 0.45% for soybeans.

In eight Brazilian bean varieties studied, with different color seed coats, a trypsin inhibitory activity of 18−29 trypsin units inhibited (TUI)/mg was found (**Table 1**), with no correlation to protein content or color of the seeds (*7*). Mancini Filho and Lajolo (*8*) reported a great variation in hemagglutinating activity for 15 Brazilian varieties studied and found a positive correlation

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Table 1. Protein Content and Trypsin Inhibitory Activity of Raw and Autoclaved (121 °C/15 min) Brazilian Varieties of Common Beans[a]

bean cultivar	% protein (N × 6.25)	raw bean (TUI[b]/mg of bean)	autoclaved (TUI/mg of bean)
Aporé	21.19 ± 0.39	18.78 ± 0.22	0.62 ± 0.01
Carioca MG	22.02 ± 0.25	28.96 ± 0.21	0.71 ± 0.01
Emgopa 201	21.03 ± 0.14	19.68 ± 0.84	1.00 ± 0.02
Jalo Precoce	25.72 ± 0.29	26.14 ± 0.93	0.56 ± 0.02
Roxo 90	22.77 ± 0.61	20.45 ± 0.62	0.69 ± 0.02
Rio Tibagi	22.36 ± 0.23	21.47 ± 0.76	0.48 ± 0.03
Safira	23.50 ± 0.55	25.55 ± 1.13	0.92 ± 0.03
Xamego	21.96 ± 0.34	26.44 ± 0.36	0.89 ± 0.08

[a] Reproduced with permission from ref *7*. Copyright 1998 SBCTA. [b] TUI, trypsin units inhibited.

Table 2. α-Amylase Inhibitory Activity of 150 Varieties of *P. vulgaris* Classified by Bean Color[a]

	specific inhibitory activity (AIU[b]/mg of protein)	
bean color	range	average
pale brown	0.16–0.40	0.29 ± 0.06
brown	0.14–0.35	0.26 ± 0.06
beige	0.14–0.40	0.26 ± 0.07
brownish	0.09–0.32	0.20 ± 0.06
dark brown	0.19–0.33	0.25 ± 0.06
black	0.11–0.30	0.19 ± 0.05
red	0.16–0.37	0.25 ± 0.05
pinkish	0.16–0.28	0.21 ± 0.05
white	0.14–0.33	0.23 ± 0.06
purplish	0.17–0.22	0.19 ± 0.02

[a] Reproduced with permission from ref *2*. Copyright 1991 Institute of Food Technologists. [b] An α-amylase inhibitory unit (AIU) value of 10 is defined as a 50% decrease in enzyme activity at 37 °C/5 min after addition of 1% starch as a substrate.

between toxicity of beans for mice and agglutinating activity toward bovine red blood cells (which varied 30 times among varieties).

The content of α-amylase inhibitors differs greatly among legumes, the highest amounts being found in *P. vulgaris* cultivars. The α-amylase inhibitor was found in common beans (*P. vulgaris*) and runner beans (*Phaseolus coccineus*) at levels of 2−4 g/kg of seed meal. In field beans (*Vicia faba*), black-eyed peas (*V. unguiculata*), and chickpeas (*Cicer arietinum*), lower levels, of 0.1−0.2 g/kg of seed meal, were reported. In lentils, soybeans, peas, sunflower seeds, winged beans, lima beans (*Phaseolus lunatus*), and adzuki beans no α-amylase inhibitor activity was detected (*4*, *9*). Screening of 150 Brazilian varieties of *P. vulgaris*, classified by bean color, revealed average values between 0.19 and 0.29 α-amylase inhibitor unit/mg of protein (**Table 2**), with no correlation between inhibitory activity and seed coat color (*2*).

NUTRITIONAL AND PHYSIOLOGICAL EFFECTS

Most of the knowledge regarding the nutritional effects of lectins and enzyme inhibitors is derived from animal experiments. There are only a few studies with human subjects. In legumes, soybean trypsin inhibitors have been more intensively studied followed by kidney bean PHA. Many of these studies have been undertaken with raw legumes or native purified proteins, a factor to be considered in the evaluation of their nutritional significance. Legumes are very rarely consumed by humans without previous heat treatment, and the effects of the consumption of individual components cannot be always related to those of a mixture, as normally present in a diet.

Protease Inhibitors. Research on the nutritional importance of protease inhibitors started with the observation that the nutritional quality of legumes could be significantly improved by heat treatment, nowadays a fact attributed to the thermal inactivation of not only trypsin inhibitors but also lectins and to improved digestibility of denatured proteins. Later, it was shown that the effect of trypsin inhibitors was not only a consequence of intestinal digestion inhibition, but also when present in diets consisting of free amino acids, a decreased growth was observed. Instead, Kunitz and Bowman−Birk inhibitors were shown to cause an enlargement of the pancreas (hypertrophy and hyperplasia) in rodents and birds and hypersecretion of digestive enzymes. This loss of the sulfur-rich endogenous proteins trypsin and chymotrypsin would result in growth depression as soy proteins are also deficient in these amino acids (*1*, *3*).

Most of these studies were done with soybeans or trypsin inhibitors purified from soybeans, in experiments with rats. Purified cowpea (*V. unguiculata*) trypsin inhibitor was also reported to provoke moderate reduction in weight gain and slight but significant depression of net protein utilization, in short-term experiments with rats (*10*). Direct infusion of the purified soybean Bowman−Birk inhibitor into the duodenum of human beings increased significantly the secretion of trypsin, chymotrypsin, and elastase by the pancreas, similar to the effects observed in rats (*3*). The mechanism of action of both trypsin inhibitors, Kunitz and Bowman−Birk, would be suppression of the negative feedback regulation of pancreatic secretion through increased release of the hormone cholecystokinin from intestinal mucosa, as reviewed by Liener (*3*).

Recently, Pustzai et al. (*11*) reported that previous complexation of the Kunitz or the Bowman−Birk inhibitors of soybeans, with trypsin and/or chymotrypsin, did not eliminate their stimulating effect on the discharge of cholecystokinin or on the pancreatic secretion in rats, suggesting that the lowering of duodenal protease levels must not be the only signal for cholecystokinin secretion.

Lectins. In short experiments, purified lectins from beans or soybeans impaired growth of rats, induced enlargement of the small intestine, caused damage to the epithelium of the small intestine, and stimulated hypertrophy and hyperplasia of the pancreas. A proximal lesion (reduction in villus cell population) with increase in enterocyte population of the jejunal villi and length, population, and crypt cell production of the jejunal and ileum crypts were observed (*12*). Reduction in maltase and invertase activities of the intestinal mucosa and interference with glycose transportation were also reported (*13*). At higher levels, bean lectins induced depletion of body and skeletal muscle, lipid, and glycogen (*14*, *15*).

Kidney bean PHA seems to influence the size, metabolism, and function of the entire gastrointestinal tract of rats, inducing a dose- and time-dependent growth of the small intestine, by lengthening the tissue and thickening the gut wall by increasing the number of crypt cells, and of the large intestine and pancreas, by hypertrophy (*16*). When present in the diet of young rats, it caused depression of growth with changes in body composition, lipid weight being the most affected independent of lectin dose. Skeletal muscle weight losses increased, in relation to body weight losses, at higher lectin doses. A depression of plasma insulin level without effect on plasma glucose level, independent of the PHA dose, was also observed. However, the muscle weight losses do not seem to be a direct PHA effect but the consequence of extensive *Escherichia coli* overgrowth in the small intestine (*15*).

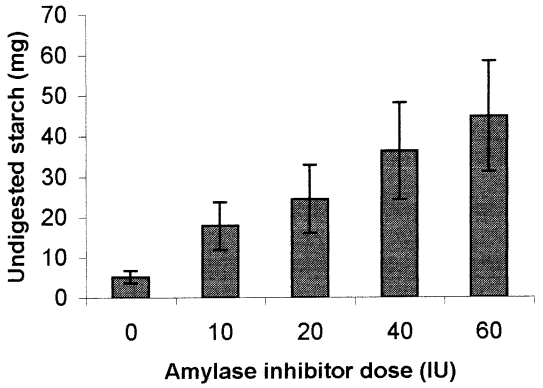

Figure 1. Undigested starch in the gastrointestinal tract of rats measured 3 h after feeding of starch plus α-amylase inhibitor by stomach tubing. IU, international units. (Based on data from ref *22*.)

The pancreatic growth in rats induced by PHA is a result of increasing cholecystokinin release, whereas intestinal growth was stimulated by an independent mechanism (*17*). The proliferative effects on gastrointestinal cells are associated with increased uptake and synthesis of polyamines. Enterocyte-like Caco-2 cells exposed to PHA had a significant increase in ornithine decarboxylase activity and polyamine content during the proliferative phase of cell growth, similar to brush border cells of the rat small intestine (*14*, *18*).

Similar to trypsin inhibitors, PHA seems to stimulate secretion of pancreatic digestive enzymes, but in a way only partially modulated through cholecystokinin, as this effect could only be partially attenuated by pretreatment of rats with a CCK-receptor antagonist (*19*).

α-Amylase Inhibitors. Studies are still needed to clarify the possible roles that α-amylases inhibitors could have in human nutrition. A few years ago, some preparations of α-amylase inhibitors from kidney beans were commercialized, but they were proven to have no effect on starch degradation and utilization by humans. This fact was explained by the instability of the inhibitor in the stomach, by the low amount of inhibitor in relation to α-amylase, and by the fact that the inhibitor was active only after preincubation with the enzyme in the absence of starch. Also, these preparations were shown to be contaminated with lectins and trypsin inhibitors (*1*). Recent results confirmed that the α-A1-inhibitor from beans inhibited porcine pancreatic α-amylase in vitro only when preincubated together, before substrate addition (*20*). This means that when consumed within a diet the inhibition would not occur.

However, in acute experiments with rats, after administration of a black bean inhibitor in a starch meal by stomach tubing, a slower starch digestion was observed (**Figure 1**), with reduction of serum glucose (**Figure 2**) and insulin concentrations and an increased metabolism of nonesterified fatty acids from the adipose tissue (*2*). These effects were also observed for diabetic rats (**Table 3**) (*21*). A reduction of calorie utilization from the diet was also observed in mid-term experiments with rats with restricted calorie ingestion (**Figure 3**) (*22*). Similarly, Pustzai et al. (*23*) reported reduced utilization of dietary starch and protein for rats in a 10 day experiment with purified α-amylase inhibitor.

INACTIVATION DUE TO PROCESSING

Because of their proteic nature, enzyme inhibitors and lectins are inactivated under the conditions leading to irreversible protein denaturation. As all food legumes are heat treated before consumption by humans, the destruction of these antinutritional factors is expected, although some residual activity can be found when proper heating was not achieved or when a balance has to be made between thermal destruction of inhibitors and deleterious effects of excessive heat on protein quality (*3*). The most common grain legume processing methods, such as cooking at atmospheric pressure and cooking under pressure, are efficient in inactivating most or even all of the protease inhibitory activity (**Table 1**). This inactivation is time and temperature dependent and is different in the grain matrix compared to the more stable purified forms (*24*, *25*). Also, the presence of thiols during heat treatment facilitates inactivation of protease inhibitors (*26*).

Lectins, similar to protease inhibitors, are inactivated by cooking beans for 15 min at atmospheric pressure or for 7.5

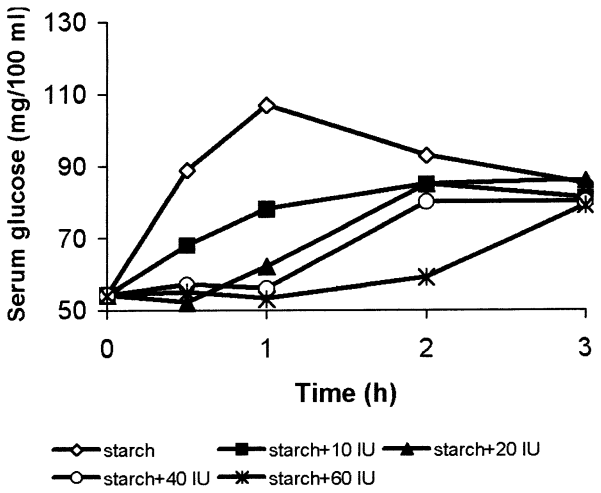

Figure 2. Dose–response relationship of serum glucose of rats measured 3 h after feeding of starch plus α-amylase inhibitor (10–60 IU/100 g of rat body wt) by stomach tubing. IU, international units. [Data from Lajolo (unpublished data).]

Table 3. Effect of the α-Amylase Inhibitor on Serum Glucose in Diabetic Rats, after Feeding Starch by Stomach Tubing[a]

	serum glucose (mg/100 mL)	
time (h)	starch	starch + inhibitor (80 IU)
0	251 ± 56	251 ± 56
0.5	317 ± 40a	249 ± 66b
1	330 ± 41a	251 ± 53b
2	358 ± 61a	261 ± 53b
4	303 ± 41a	270 ± 54b

[a] Based on data from ref *21*. a ≠ b ($p \leq 0.05$).

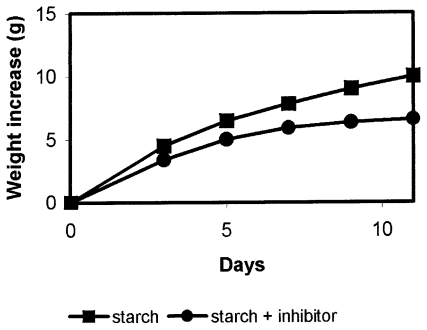

Figure 3. Effect of the α-amylase inhibitor (1 mg/rat/day) on growth of rats fed a calorie-restricted diet (4 kcal/rat/day). [Data from Lajolo (unpublished data).]

min under pressure cooking. However, they seem to be more resistant in ground bean or soy flours and also to dry heat treatments as compared to moist heat (*3*, *27*). Armor et al. (*28*) reported complete inactivation of soy lectin and protease inhibitory activity by heat treatment of imbibed seeds at 100 °C for 10 min.

Extrusion was also shown to be effective in reducing trypsin inhibitory activity in beans (*29*) but ineffective in reducing phytohemagglutining activity (*30*).

Amylase inhibitors are also inactivated by thermal processing, although there are only a few studies about this subject. Cinco et al. (*31*) and Iguti and Lajolo (*32*) reported different thermal stabilities between two isoforms of α-amylase inhibitors in *P. vulgaris* beans, one being much more resistant to treatment at 70 °C. However, there is no indication of a possible survival after heating at the temperatures normally used for cooking beans, 100 °C (atmospheric pressure) or 121 °C (under pressure). Grant et al. (*9*) observed that the α-A1 activity present in kidney beans was completely abolished by cooking imbibed beans at 100 °C for 5−10 min.

RESISTANCE TO PROTEOLYTIC DEGRADATION

Residual antinutritional factors in the human diet can be of concern if they are able to survive passage through the gastrointestinal tract, to exert their biological activity. There are several reports of resistance to digestion of lectins, trypsin, and α-amylase inhibitors, even in the denatured form (*33*, *34*). However, there is no need of complete proteolysis for the loss of biological activity. A cranberry bean α-amylase inhibitor, found to pass through the small intestine of rats without loss of antigenicity, resistant to in vitro pepsin and trypsin digestion, was shown to be rapidly inactivated by chymotrypsin, although only partially hydrolyzed, probably as a result of cleavage near the active site (*35*, *36*). A purified α-amylase inhibitor from white kidney beans, resistant to pepsin digestion, was recently shown to be readily digested by physiological amounts of kidney homogenate, plasma proteases, Pronase, or thermolysin (*37*).

There is also some disagreement between data from in vitro and in vivo susceptibilities to proteolysis. The Kunitz inhibitor from soybeans is reported as being completely inactivated by human gastric juice, whereas the Bowman−Birk inhibitor remains active (*1*, *3*). Bowman−Birk inhibitors from dry beans were also shown to be resistant to in vitro pepsin, trypsin, and chymotrypsin digestion (*4*, *33*). Tsukamoto et al. (*38*) reported maintenance of inhibitory activity even after 3 h at pH 2 with a pepsin/inhibitor ratio of 1:1. Contrary to these results, Hajós and Gelencsér (*39*) found that ∼76% of the soybean Kunitz inhibitor survived passage through the small intestine of rats, whereas most of the Bowman−Birk inhibitor was digested. Similarly, cowpea trypsin inhibitor (Bowman−Birk family) was rapidly broken down in the digestive tract of rats (*10*).

The effective binding of lectins to surface receptors of the epithelial cells of the gut is a result of their resistance to proteolysis in the gastrointestinal tract, remaining in a biologically and immunologically intact form (*16*). Also, ∼8% of soybean agglutinin was found intact in a free form, in addition to the amount bound to the epithelium, in rats (*39*).

EFFECTS OF CHRONIC INGESTION OF LOW LEVELS

The physiological and nutritional significance of chronic ingestion of low residual inhibitor and lectin levels remains an open question. In relation to trypsin inhibitors, it is known that soy products retain 5−20% of the trypsin inhibitory activity originally present in raw soybeans, as a consequence of thermal treatments not intended to damage functional and nutritional properties of proteins (*3*). However, the methodology used to measure residual activities has not been considered to be precise in most cases, including the fact that inhibitory activity is generally measured against bovine and not human proteases. Data on specific inhibition against human enzymes are more scarce. According to Belitz and Weder (*1*), the average daily amounts of trypsin and chymotrypsin produced by humans (1−2 g of each) could be completely inhibited by the ingestion of 100 g of raw soybeans, or 200 g of lentils or other legumes, containing 2 g of inhibitor. Soy-based infant formulas would inhibit 0.05−0.1 g of human trypsin and 0.01−0.02 g of human chymotrypsin per day. Burns (*40*) reported that processed soy products would have ∼3% of trypsin inhibitory activity of the raw flour, equivalent to the inhibition of ∼2−5 μg of trypsin/mg of protein, amounts of no nutritional or physiological concern in rats. However, there are reports on long-term feeding experiments with rats showing an increase in the probability of pancreatic adenoma formation (*1*). The average British diet would provide 0.3 g of bovine trypsin inhibitor per day, 65% of this from eggs, milk and milk products, and potatoes (*41*). According to Deshpande (*42*), in developing countries, where there is a higher contribution of legumes in the diet, there would be a lower protease inhibitor uptake. Trypsin inhibitor activity in eight cultivars of *P. vulgaris* beans, normally present in the Brazilian diet at levels of 60−70 g/person/day, was shown to be completely inactivated after cooking at 121 °C for 15 min (*7*). However, between 2 and 5% of residual activity was detected by the colorimetric method of Kakade et al. (*43*) (**Table 1**), and the same was observed with soy products, including soy-based infant formulas, a problem that was related to nonspecific interferents (*44*).

In relation to lectins, there seems to be no residual activity left in properly processed legumes. Also, hemagglutinins in legumes were shown to be inactivated before they were considered to be edible (*42*). The same seems to apply to α-amylase inhibitors. However, Liener (*3*) pointed out that lectins are more resistant to dry heat treatment, and products such as cereal-type food and cookies containing soy flour as an ingredient showed some lectin activity, as did soybean oil. The author, however, thinks it highly unlikely that this level would pose any risk to humans. In this sense, Deshpande (*42*), in an interesting discussion of available data on food legumes, concluded that there is enough evidence indicating their safety for human nutrition.

NUTRITIONAL UTILIZATION OF PROTEIN INHIBITORS AND LECTINS

Besides their physiological effects, another important fact is that protein molecules of trypsin inhibitors are very poorly digested, making their high cystine content unavailable, a question of concern considering legume sulfur amino acids deficiency (*45*, *46*). According to Kakade (*45*), although trypsin inhibitors comprise only ∼2.5% of total bean proteins, they would contribute 32 and 40% of the total cystine of lima and navy beans, respectively. Similarly, lectins and α-amylase inhibitors were shown to be glycoproteins resistant to digestion (**Table 4**) and, together with trypsin inhibitors, participate in chemical reactions during processing, reducing overall protein digestibility, a still unsolved question (*34*, *47*−*49*). The main storage protein of dry beans, phaseolin, is completely digested after thermal treatment. On the contrary, the albumin fraction, which concentrates α-amylase inhibitors, trypsin inhibitors, and

Table 4. Pepsin–Pancreatin Digestibility of Lectins and α-Amylase Inhibitors from Common Beans[a]

	% hydrolysis	
	3 h pepsin	3 h pepsin + 4 h pancreatin
α-amylase inhibitor I		
native	9.5	17.3
heated	9.8	24.1
α-amylase inhibitor II		
native	9.2	17.7
heated	10.0	22.0
lectins		
native	9.4	11.6
heated	13.7	31.5
albumins		
native	20.4	32.0
heated	11.6	14.3
phaseolin		
native	9.8	22.7
heated	27.2	84.5
casein	25.4	86.7

[a] Reproduced with permission from ref *34*. Copyright 1996 Food and Nutrition Press, Inc. Results were the mean of two determinations. Differences between values were <10% of the mean. Aqueous solutions of proteins (10 mg/mL) were heated at 99 °C for 30 min and submitted to pepsin–pancreatin digestion.

lectins, causes an increase in exogenous and endogenous N excretion (*50*, *51*). This fact has to be considered in the development of new legume varieties resistant to insects, on the basis of a higher content of these plant defense substances. For instance, for diets containing up to 30% transgenic peas, with a higher content of recombinant α-amylase inhibitor A1, minimal detrimental effects on the nutritional value for rats were observed. However, at higher inclusion levels, a decrease of nutritional value was reported (*52*). In the case of lectins, another important fact is that these proteins have no methionine and a very low content of half-cystine, and an increased content in the seed will probably represent a decrease in nutritional value due to an exacerbation of sulfur amino acids deficiency.

POSSIBLE USEFUL EFFECTS

Lectins seem to be promising biologically active substances, and their exploration for possible medical use seems to be very attractive, as a result of their abilities to provoke hyperplasia of the small intestine, induce changes in its bacterial flora, interfere with hormone secretion, and enter the systemic circulation (*53*, *54*). Much evidence has been recently obtained on their possible beneficial effects as biomedical agents.

The inclusion of purified bean phytohemagglutinin in the diet of mice reduced tumor growth rate in a dose-dependent manner, suggesting a competition for nutrients between the gut epithelium undergoing hyperplasia and the developing tumor (*55*).

Lectins have also been suggested as agents to prevent gastrointestinal atrophy during total parenteral nutrition, alone or in combination, as different lectins seem to act in different regions of the gastrointestinal tract. In rats, PHA administered intraluminally increased proliferation in the gastric fundus and in the small intestine, indicating that it could be associated with peanut agglutinin, the effects of which were observed in the large intestine (*56*). The same could be said in case of surgical removal of part of the small intestine (*12*).

In obese rats, a reduction of lipid accumulation by inclusion of raw kidney bean in the diet was observed, which was related to a decrease of insulin levels caused by lectins. However, no body or muscle protein losses occurred, even at high doses, as with normal rats, suggesting a possible use of lectins as therapeutic agents to treat obesity (*57*).

Bowman−Birk inhibitor has been shown to be effective at preventing or suppressing carcinogen-induced transformation in vitro and carcinogenesis in animal carcinogenesis assays and achieved Investigational New Drug status from the U.S. FDA in 1992 (*58*−*60*). Recently, some studies with human beings were initiated (*61*, *62*). Besides the anticarcinogenic effects, Bowman−Birk inhibitor also showed anti-inflammatory action, by inhibiting proteases released from inflammation-mediating cells and suppressing superoxide anion radical secretion from immunocytes. Recently, a reduction of ulcerative colitis in mice was reported, suggesting that these inhibitors could have other therapeutic actions in humans (*60*, *63*).

It must be considered that the potential beneficial effects require the native proteins and therefore would not be attained after proper cooking of legumes.

CONCLUSIONS

From the above discussion it seems clear that lectins and enzyme inhibitors present in the diet do not pose health risks to humans or cause antinutritional effects in normal conditions of consumption, that is, after proper thermal processing, even after chronic ingestion of low residual levels. Their main antinutritional effect seems to be their resistance to digestion, due to structural problems and reactions occurring during processing, leading to increased secretion and excretion of N and S from the body and as a consequence low availability of sulfur amino acids. Consequently, increased contents of these substances in seeds should be viewed cautiously. On the other hand, they seem to have important pharmacological effects that should be confirmed, the mechanisms of which need to be established in order to be used.

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