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REVIEW ARTICLE

Anti-Nutritional Factors in Foods and their Effects

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Abstract

Plants commonly synthesize a range of secondary metabolites as part of their protection against attack by herbivorous, insects and pathogens or as means to survive in adverse growing conditions. If farm or domestic animals or humans consume these plants, these compounds may cause adverse physiological effects. The term anti-nutrients refers to defence metabolites, having specific biological effects depending upon the structure of specific compounds which range from high molecular weight proteins to simple amino acids and oligosaccharides. Legumes are rich source of anti-nutrients in human diet. This review will focus on phytic acid, saponins, polyphenols, lathyrogens, alpha amylase inhibitors and lectins which are found in grains and legumes. Anti-nutrient substances from nutritional point of view, interferes with normal growth, reproduction and health, when consumed regularly in amount existing in a normal component of diet therefore should be considered as harmful and toxic. A significant part of human population relies on legumes as staple food for subsistence, particularly in combination with cereals. They are unique foods because their rich nutrient content includes starch, protein, dietary fibre, oligosaccharides, phytochemicals (especially the isoflavones in soybean) and minerals. Their nutritional content contributes to many health benefits to humans. So, the knowledge regarding various anti-nutritional substances present in foods as well as techniques to reduce them in the diet is essential for health and well being of the population.

Keywords: Anti-nutrients, saponins, polyphenols, phytochemicals, dietary fibre, germination.

Introduction

To avoid predation, sedentary species (plants, fungi and bacteria) synthesize a range of low and high molecular weight compounds. These secondary metabolites play a role in defence against herbivorous, insects, pathogens or adverse growing conditions (Herbourne, 1989). In India, people are mostly vegetarian, depending largely on cereals and pulses, as their staple foods provide the main source of dietary proteins and calories (Khokhar and Chauhan, 1986). Legumes commonly used as foods includes soybeans (*Glycine max*), black gram (*Phasedous mungo*), cow pea (*Vigna unguiculata*), dry beans (*Phaseolus vulgaris*), winged beans (*Psophocarpus tetragonolobus*), chick pea (*Cicer arientinum* L.), horse gram (*Doliches biflorus*), moth bean (*Vigna aconitifolia*), pigeon pea (*Cajanus cajan*), favabeans (*Vicia faba* L. minor), grain amaranth (*Amaranthus* spp.), lentil (*Lens culinaris* medic), jackbean (*Canavalia gliadata*) and grass peas (*Lathyrus sativus*) (Ahn *et al.,* 1989). Legumes contain higher percentages of protein than cereal and provide a relatively affordable protein source in developing countries. Physical and chemical methods are employed to reduced or remove anti-nutritional factors including soaking, cooking, germination, fermentation, selective extraction, irradiation and enzymic treatment. Application of single technique is frequently insufficient for effective treatment and so combinations are commonly employed.

Industrial processes, including canning, toasting, fractionation and isolation of protein concentrates have been shown to be effective in reducing or removing anti-nutritional factors. However, it should be borne in mind that processing can also introduce undesirable compound for example volatile aldehydes and ketones and peroxides as a direct result of lipid oxidation or reduce levels of desirable compound ex-proteins and essential minerals (Khokhar and chauhan, 1986). The proteins from pulses are known to be inferior quality, due to the deficiency of sulphur containing amino acids as well as due to other factors like digestibility, availability of amino acids, anti-nutritional factors, etc. The anti-nutritional factors present in this type of food, including their contents of enzyme inhibitors, lectins, flatulence factors, tannins, phytic acid and saponins (Price *et al.,* 1987). Anti-nutritional factors can cause detrimental effects to humans and animal growth and performance by impairing intake, uptake or utilization of other foods and feed components or by causing discomfort and stress to humans and animals. The anti-nutritional factors mainly occur in pulses and grain legumes and foods and feed material prepared from grain legumes and pulses (Friedman, 2001). Anti- nutritional factors in grain legumes can be divided into several groups based on their chemical and physical properties such as non-protein amino acids, quinolizidine alkaloids, cyanogenic glycosides, pyrimidine glycosides, isoflavones, tannins, oligosaccharides, saponnins, phytates, lectins or protease inhibitors. Their elimination can be achieved either by selection of plant genotype with low levels of such factors or through post-harvest processing (germination, boiling, leaching, fermentation, extraction etc.). Structure of anti-nutritional factors and their chemical properties especially heat lability, dictate which physical process will be more effective in their reduction or removal, thereby minimizing adverse biological effects (Shahidi, 1997).

Positive effects of anti-nutritional factors in human nutrition

The anti-nutritional factors found to have effect on gastrointestinal tract and affect the microflora count of the intestine by promoting the growth of beneficial bacterias. *Lupinus campestris* seed found to have anti-mutagenic activity and prevents the mutagenic process involved in development of cancer. Anti-nutritional factors decreases levels of heat shock protein 70 and 90 in gut epithelial cells after exposure to plant lectins. Lectins present in legumes assessed to act as a mucosal adjuvant. Beneficial outcome in hypercholesterolemia after intake of heat treated chickpea in rats have been observed (Price *et al.,* 1987; Jansman *et al.* 1998; Friedman, 2001; Young, 2011).

Negative effects of anti-nutritional factors in human nutrition

Low toxic substances in legumes produce serious pathological conditions. They are the factors in kesari dhal which cause lathyrism and haemolytic factor in *Vicia faba* associated with disease favism.

Lathyrism: Lathyrism is a paralytic disease affecting the lower limbs. The incidence of the disease is higher in males than females and recovery from the condition does not usually occur. The disease has been known since early times and there is reference to it in early Indian medical writing. Serious outbreak of lathyrism has occurred in this country quite a few times. The disease has been associated with consumption of kesari dhal and is commonly noticed in poor families who regularly eat considerable quantity of the dhal. However, lathyrism develops only when the consumption of dhal is high (300 g daily) and the diet does not contain adequate quantities of cereals and is used for long time (six months or more). In lathyrism, the toxic substance interfaces with the formation of normal collagen fibre in the connective tissue. The disease can be prevented by ensuring reasonable balance between kesari dhal and other material and its replacement by other pulses where practicable. Lathyrogens i.e. Beta N oxayl, Beta diamino propionic acid (BOAA), a naturally occurring amino acids, possess potent neurotoxic activity and has been shown to be responsible for outbreaks of neurolathyrism following consumption of *Lathyrus sativus*. BOAA occurs naturally as two isomeric forms with the Beta N oxayl L form being approximately 5% of the total. The level of BOAA in dry seeds varies considerably according to genetic factors and environmental conditions. *Lathyrus sativus* grown in nutrient solution that are zinc deficient or rich in ferrous iron, produced seeds with elevated levels of BOAA (Burbano,1999).

Favism: Favism is a disease characterized by haemolytic anaemia which affects certain individuals following the ingestion of fresh or cooked broad beans. The victims suffer from an inherited biochemical abnormality which affects the metabolism of glutathione in red blood cells and is the result of decreased activity of the enzymes glucose-6-phospate dehydrogenase. In person with his abnormality, the red cells are more prone to injury and destruction by certain drugs, such as sulphonamide and this raises complications in the treatment of infectious disease (Dmello *et al.,* 1991).

Kinds of anti-nutritional factors

There are several heat stable and heat labile anti-nutritional or toxic and potentially toxic factors. These include trypsin inhibitor, phytohaemaggluttins, goitrogens, cynogenetic glycosides, antivitamin factors, metal binding constituents, estrogenic factors, toxic amino acids, lathyrogens, flavogens and unidentified growth inhibitors. These factors in seeds of many legumes and cereals could give rise to problems in nutrition. Some details of the anti-nutritional factor are given hereafter.

Protease (Trypsin) and amylase inhibitors: Protein modification by enzymes yields products with improved nutritional, functional and organoleptic properties and aids a variety of processing operations. Proteinases are used by the food industry to control viscosity, elasticity, cohesion, emulsification, foam stability and whipability, flavour development, texture modification, nutritional quality, solubility, digestibility and extractability. Compared with legumes, common anti-nutrients level in cereal are quite low. Although digestive enzyme (protease and amylase) inhibitors have been identified in most cereals, they do not pose serious nutritional problems. Rye, triticale, and barley contain higher levels of protease (trypsin) inhibitor activity than other cereals. In high- lysine corn barley, that activity is higher than in the corresponding normal varieties. Bread wheat has nearly twice as much trypsin inhibitor activity as other wheat. However, that activity is much lower in cereals than in legumes. For example, total protein inhibitor in barley normally is about 0.45 g/kg, whereas in defatted soy flour it is more than 32 g/kg (Dmello *et al.,* 1991). Application includes processes for meat flavour development and tenderization, continues bread making and modification of cracker and cookie texture, malt supplementation and chill proofing in brewing industry, and hydrolysis of protein gel to lower viscosity for concentration or filteration. The desired degree of hydrolysis or percentage of peptide bonds hydrolyse varies considerably with the different food processing operations.

Some proteolytic processes, such as for bouillcn from soy protein or fish sauce from whole fish, require a degree of hydrolysis close to 100%. In contrast, in many food processing operations, there is a balancing act in which just enough, but not too much protein hydrolysis must be achieved. Among enzymatic food protein modifications, “limited hydrolysis” is a technique receiving considerable attention because it can yield products with improved properties and added value (Jansman *et al.,* 1998).

Haemagglutinins: Haemmagglutinins are proteins in nature and are sometimes referred to as phytoagglutinins or lectins. As in legumes, most cereals commonly consumed by human contain glycoprotein called lectins. Many lectins can bind to intestinal epithelial cells, where they may impair nutrient absorption and cause damage that may allow infiltration of bacteria into the blood stream. Although considerable indications are there and these legume lectins can be harmful to humans, virtually no evidence exits of any significant anti-nutritional effect from cereal lectins (Jansman *et al.,* 1998).

Saponins: Saponins occur widely in plant species and exhibit a range of biological properties, both beneficial and deleterious. Saponins are group of natural products possessing the property of producing lather or foam when shaken with water. These are glycosides of high molecular weight. Saponins have been reported in soyabean, sword bean and jack bean. Toxic saponins cause nausea and vomiting. These toxins can be eliminated by soaking prior to cooking. Alkaloids are known to occur in seeds of many legume but they are relatively innocuous. The saponins can be considered as the resistant factor in legumes against microbial infection and herbivory. Legume saponins have only moderate toxicity and present a problem only when present in diet in higher concentrations (Jansman *et al.,* 1998). These are diverse class of glycosides found mainly, but not exclusively in plants, they comprises a steroidal or triterpene aglycone linked to one, two or three saccharide chains of varying size and complexity via ester and or ether linkages. Change in saponin structure, perhaps as a result of partial hydrolysis during processing, will therefore have significant effects on the quality of the processed products. Saponins occurs in broad range of plants consumed in the human diet, including legumes (soy, peas and beans), root crops (potato, yam, asparagus and allium) as well as in oats, sugarbeet, tea and many medicinal herbs such as ginseng. Within grain legumes, the saponin content varies between 0.5% and 5% dry weight. Saponins are generally characterized by their bitter taste, their ability to foam in aqueous solution and their ability to hemolyse RBC. Ingestion of saponin containing foods by man and animals has been associated with both deleterious and beneficial effects, for example reduced weight animals and hypocholesterolemic effects in man. Following oral administration, saponins are only poorly absorbed and are either excreted unchanged or metabolized in the gut (Price *et al.,* 1987).

Phytates: Inositols with 4, 5 or 6 phosphate groups are common in the seed of many of our grain legume and can reach concentration higher than 10% of dry matter (Bisby *et al.*, 1994). In monocotyledons such as wheat and rice, phytates is present in germ of corn and in the aleurone or bran layer allowing an easy separation by milling. However, in diacotyledons seeds such as legumes, nuts and oilseeds, phytates are found closely associated with proteins and is often isolated or concentrated with protein fraction of these foods. They can be regarded as stores for phosphate and mineral nutrients that are important for plant nutrition and especially vulnerable during germination (Jansman *et al.,* 1998). Since, phytates contains complex zinc, iron, magnesium and calcium ions in the digestive tract, they can cause mineral ions deficiency in animals and human. Again, these compounds seem to serve a double response i.e. defence and phosphate and mineral store. Phytate contain of food can be lowered by addition of enzymes which hydrolyze them.

Oligosaccharides and Isoflavonoids: Legume seeds are generally rich in oligosaccharides (up to 20%), such as stachyose and raffinose. These compounds serve as carbon source during germination therefore, their contents can be reduced in legumes through germination which is common practice, e.g. in soyabeans. Isoflavonoids have been detected in soyabean, lupins and several other legumes. They are involved in plant defence against fungi, bacteria, viruses and nematodes (phytoalexins, phytoanticipins), act as signals in Legume-Rhizobium interaction and exhibit estrogenic activities (Herbourne, 1989). Recently it was found that the isoflavone genistein inhibits tyrosine kinase. Since, these enzymes are often stimulated in cancer cells, the lower incidence of some kinds of cancers in people which ingest isoflavone rich food, such as soybean products, has stimulated the hypothesis, that some legumes rich in isoflavones can prevent cancer (Dmello *et al*., 1991).

Cynogenic glycosidases: Cynogens are glycosides of 2-hydroxyl nitriles and widely distributed among plants, e.g. the Rosaceae, Leguminosae (e.g., in *Phaseolus lunatus* and *Vicia sativa*), Graminae, and Araceae. In case of emergency, when plants are wounded by herbivores or other organisms, the cellular compartmentation breaks down and cynogenic glycosides come into contact with active B-glucosidase, which hydrolyses them to yield 2-hydroxynitrile. In addition to the toxic effects, cynogens can serve as mobile nitrogen storage compounds in seeds which are important during germination. Cassava is a crop plant rich in cyanogenic glycosides.

Since farmers in marginal areas rely on “bitter” varieties for food productions, it has been suggested that the prevention of cynate poisoning through improved detoxification procedures may be more effective than the development of “low cynide” cultivars (Tylleskar *et al.,* 1992).

Lupin alkaloids: Grain legume, high in protein is commonly used for livestock feed for Europe, Africa, Australia and Asia. Also used in cereals, baby formula, pasta, soups and salads in Unites States (22 states, 42,000 pounds/year). Seeds are used in traditional Chinese medicines. It has been cultivated for over 2000 years. Over 500 species of the genus Lupinus are known. In its raw form, the mildly toxic lupin alkaloids present in plants causes a bitter taste, and used as defensive mechanism herbivorous (Herbourne, 1989). Alkaloids are commonly removed (or reduced) by soaking the raw seeds in water prior to use. In the 1929’s German plant breeder produced the first alkaloid-free, “sweet seeds”. Cystinine has been identified as selective partial nicotinic receptor against (nicotinic acetylcholine receptors are affected by Parkinson’s and Alzheimer’s diseases) (Nicotine is fully against neuronal nAChR’s, and has additional undesirable biological effects). Alkaloid extracts from Lupinus species have shown antimicrobial activity (Jansman *et al.,* 1998). *Lupinus albus* showed inhibitory effect on Gram negative bacteria. Lupinus *varius* and *L. densiflorus* strongly inhibited Gram positive bacteria. Many known lupin alkaloids showed significant antifungal activity. Matrine has shown anti-ulcerogenic and anticancer activities.

Non-protein amino acids: Hundreds of types of non-protein amino acids have been found in nature and they have multiple functions in living organisms. Microorganism and plants can produce uncommon amino acids. In microbes, examples include 2-aminoisobuytric acid and lanthionine, which is sulphide-bridged alanine dimer. Both these amino acids are found in peptidic l antibiotics such alamethicin. While in plants, 1-Amino cyclopropane-1-carboxylic acid is a small distributed cyclic amino acid that is a key intermediate in the production of the plant hormone ethylene. In humans, non-protein amino acids also have biologically-important roles. Glycine, gamma-amino butyric acid and glutamate are neurotransmitter and many amino acids are used to synthesize other molecules, for e.g. Tryptophan is a precursor of the neurotransmitter serotonin, Glycine is a precursor of porphyrins such as heme, Arginine is a precursor of nitric oxide, Carnitine is used in lipid transport within a cell, Ornithine and S-adenosyl methionine are precursors of polyamines and Homocysteine is an intermediate in S-adenosylmethionine recycling (Saluanke, 2006). More than 900 non-protein amino acids (NPAAs) which are especially abundant in certain legumes (Vicieae, Phaseoleae, Mimosoideae and Caesalpinioideae) have been detected which resemble protein amino acids (structural analogues). Concentration in seeds can exceed 10% of dry weight and since non-protein amino acids are remobilised during germination, they certainly function as N-storage compounds. If non-protein amino acids are taken up by herbivorous, microorganisms or other plants, they may interfere with several targets. In plants, microorganisms and insects, non-protein amino acids cause reduced growth or even death. Since non-protein amino acids affect a basic target present in all organisms, they are important in plant–plant, plant-microbe and plant–herbivore interactions (Shahidi, 1997).

Phenolic compounds: Phenolic and polyphenolic compounds in foods and natural neutraceutical products represents the most widely distributed plant secondary metabolites exerting their beneficial effects as free radicals scavengers and chelators of pro-oxidant metals and thus preventing low–density lipoprotein oxidation and DNA strand scission or enhancing immune function. Phenolic compounds have been shown to control certain types of cancer, cardiovascular disease and process of ageing (Saluanke, 2006). Polyphenols are secondary plant metabolites distributed ubiquitously within plant foods (vegetable, cereal, legumes, fruits, nuts and beverages like tea, wine, cocoa) (Table 1). These levels vary greatly even between cultivars of the same species. Environmental factors such as light, germination, degree of ripeness, variety, processing and storage, genetic factors can influence levels (Ahn *et al.,* 1989).

Table 1. Polyphenolic content of different plant foods and beverages (Source: Bravo, 1998).

Foods	mg/Kg dry matter
Cereals	22-102.60
Legumes	34-1710
Nuts	6.04-38
Vegetables	6-2025 (mg/fresh matter)
Fruits	2-1200 (mg/fresh matter)
Tea	150-210 (mg/200 mL)
Red wine	1000-4000 (mg/200 mL)
White wine	200-300

Alpha galactosides: Flatulence causing oligosaccharides, chemically described as alpha galactosides are present in significant amounts in mature legume seeds (Khokhar and Chauhan, 1986).

Anti-vitamin factors

Raw kidney beans contain anti-vitamin E that produces necrosis of liver and muscular dystrophy. Linseed contains an anti-pyridoxine factor that depresses growth. The factor responsible is L-amino acids, D-proline that occurs as peptide linatine in combination with glutamic acid. L-amino-D-proline is about 4 times as active as linatine (Saluanke, 2006). The factor could be eliminated by extracting the meal with water. There are various methods to reduce anti-nutritional factors from food and are described in the following sections.

Table 2. Heat labile anti-nutritional factors inactivated by thermal processing (Source: Khokhar and Chauhan, 1986).

Anti-nutritional factor	Common food sources	Effects of anti-nutritional factors
Avidin	Egg whites	Binds biotin, making it biologically unavailable
Hemagglutinnins	Red kidney beans, yellow wax beans	Induces red blood cell clumping
Lathyrogens	Chick pea	Disrupts collagen structure
Goitrogens	Sweet potatoes, beans, cabbage, turnips	Causes goitre by limiting iodine absorption
a-Amylase inhibitors	Cereal grains, peas, beans	Slows starch digestion
Trypsin inhibitors	Legumes, egg whites, potatoes	Inhibits activity of trypsin
Thiaminases	Fish, shellfish, brussel sprouts, red cabbage	Destroys thiamin

Thermal treatment: Legume seeds are hardly been consumed raw, they are usually cooked and by this procedure, lectins and protease inhibitors are inactivated. Low molecular weight compounds are leached out into cooking water, to be discarded afterwards. All these simple techniques have been invented by man (even without a profound knowledge of underlying toxicology) to make legume seeds more palatable and digestible. Today, a deeper knowledge of chemical structure of the anti-nutrients can help to devise technological strategies to process legumes seeds in order to obtain toxin free products. Since a diversification will increase the economic value of overall crop plant, food technology and rational processing are an alternative to breeding of anti-nutritional factors free plants which can be more susceptible to pests and pathogens. Followed by modern food technology (separation, filteration etc.), pure nutritionally valuable dietary products, such as protein, dietary fiber, oil and other fine chemicals can be generated. The remaining fractions containing the anti-nutrients don't need to be discarded, some of them are useful for the pharmaceutical industry; others might be used in agriculture as biorational pesticides (Jansman *et al.,* 1998). Because of higher costs these considerations are related to the use of legumes seeds which will be utilized for human consumption (an example are soyabeans for which a specialized industry has been developed). These situations is different in case of feed industry (which is a major outlet of legume seeds in Europe) where pulses are simple ground or pelleted. For this purpose we need to develop varieties which are low in heat stable nutritional factors (e.g. alkaloids, saponins, phytates, isoflavones and non-protein amino acids). If heat labile compounds (protease inhibitors, lectins) can be denatured by heat treatment during grinding and pelleting these compounds might be maintained since they confer resistance to the plants. Thus, many fields remain to be explored even in widely known group like the temperate grain legumes (Khokhar and chauhan, 1986). Some of the heat labile anti-nutritional factors inactivated by thermal processing are given in Table 2.

Chemical detoxification: Deaminocanavanine is a well known untoxic deamination product of canavine. The degradation of canavanine to deaminocanavanine under alkaline conditions occurs therefore a chemical strategy for the detoxification of this compound and has already been successfully employed for the processing of the canavanine containing seeds of *C. ensiformis*. The major anti-nutrients in the seeds of *V. sativa* (besides vicine) can be rendered inactive by mild hydrolysis. It is reasonable to propose that in principle post-harvest detoxification procedures can be developed for these anti-nutritional factors (Janseman *et al.,* 1998).

Fermentation: The use of fermentation as an integral part of food detoxification processes is widely practiced. A wide variety of fermented foods are produced and eaten around the world. Fermentation is also an effective means for food preservation. Fermented foods can be prepared at both, an industrial and household scale. Indeed, many fermented foods are prepared by very simple techniques and represent grass roots technology which is already widespread; a fact which facilitates their further refinement, transfer and adoption in underdeveloped countries for the detoxification of alternative food sources. The various Indian household preparation techniques for their effectiveness in detoxifying *L. sativus* and their methods which included a fermentation step were the most effective in reducing ODPA levels, eliminating 95% of this toxin (Khokhar and Chauhan, 1986). Further, improvement in detoxifying is likely to be made with selection for better ODAP degradation. Such methods can, in principle, also be used for the post harvest detoxification of Vicia seeds, thus providing an alternative approach to the wider utilization of these grains without the need for genetic removal of their low molecular weight anti-nutritive and unpalatability factors. The incorporation of fermentation processes into other simple food technologies also offer good prospects for a detoxification of food source while simultaneously giving flexibility in the manipulation of flavour, texture and colour of the raw material (Jansman *et al.,* 1998).

Germination: Pea and lentil sprouts have gained popularity in recent years. Traditionally, Mediterranean, grain legumes have not been used as sprouts. The potential toxicity of beta-isoxazolin-5-one-alanine (BIA), the biosynthetic precursor for the lathyrism toxin beta-ODAP may be a risk factor if consumption increases during the germination of lentils and peas. This kind of processing, however, which reduces the contents of oligosaccharides and of other N-containing ANFs, has a long history in Asia, where it has served to improve the palatability of soyabeans. Pea sprouts are a very recent addition to Chinese cuisine (Borade *et al.,* 1994).

Conclusion

The development of new food crops from Lupinus, Vicia and Lathyrus species is used to illustrate the problems associated with heat stable low molecular weight anti-nutritional factors. These substances include proteolytic inhibitors, phytohemagglutinins, lathyrogens, cynogenetic compounds, compounds causing favism, factors affecting digestibility and saponins. These factors are shown to be widely present in leguminous foods which are important constituents of the diet of a large section of the world's population, and particularly, of people in the developing countries. Knowledge regarding ways and techniques to lower down or reduce the content of anti-nutritional factors in food is needed for health and well being of the population.

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