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ANTI-NUTRITION IN LEGUMES: EFFECT IN HUMAN HEALTH AND ITS ELIMINATION

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ABSTRACT
In India, legume seeds are identified as a major source of protein after milk. Bean (soybean, cowpea, French bean, boad bean, lima bean etc.) seeds have a unique nutritive value as they deliver valuable proteins, saccharides, minerals and vitamins, and dietary fibre. Besides, they contain a wide range of bioactive compounds that cannot be considered as nutrients; however they exert physiological effects on humans. These may reduce the availability of otherwise good proteins in the diet and cause diseases originating from malnutrition. These factors affecting digestibility include proteolytic inhibitors, phyto hem agglutinins, phytic acids and tannins etc. 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 people in the developing countries.

Introduction
Legumes produces comparably large and protein-rich seed which often contain substantial amount of "anti-nutritive" factors (ANF), such as lectins, protease inhibitors, non-protein amino acids (NPAAs), alkaloids, cyanogenic glycosides, pyrimidine glycosides, saponins, tannins, isoflavones, oligosaccharides, erucic acid, or phytates. Since many of the ANFs are either toxic, unpalatable or undigestible. These can be eliminated by thermal heat, cooking, soaking treatment etc.
Today several more strategies are available to minimise the impact of ANFs in grain legumes in order to improve their utilisation:

- Reduction of ANFs through processing (germination, boiling, leaching, fermentation, extraction, etc)
- Reduction of ANFs through genetic manipulation (selection from natural or artificial diversity, genetic engineering of biosynthetic pathways or of the toxic protein itself)
- Improvement of tolerance to ANFs through supplementation of diets with protective factors e.g., methionine, threonine, feed enzymes

Table 1. Toxic substances in some legume vegetables

Sl. No.	Legumes	Toxic compounds	Adverse effect
1.	Legume vegetables	Lectins, Cyanogenic glucosides, Trypsin Inhibitors	Allergens
2.	Soybean	Trypsin Inhibitors	Prevent proteolytic enzyme activity

3.	Peas and Beans (Mature seeds)	Trypsin Inhibitors, Phytic acid (Inositol hexaphosphoric acid), Antivitamin E- factors	Render Ca and Fe unavailable
4.	Cowpea	Alpha amylase Inhibitors	
5.	Dolichos bean	Trypsin Inhibitors	
6.	French bean	Flatylence causing factor, Vicin and covicine, haemaglutine	
7.	Broad bean	Haemolytic factor causing 'favism' (Haemolyticanaem ia)	
8.	Lima bean	Difficult to digest starches and cyanogenic glucoside	
9.	Soybean, Sward bean and Jack bean	Saponins	

In order to survive, plants have developed defences against herbivorous animals, microorganisms and virusesduring evolution. Furthermore, plants compete with other plants for light, water, and nutrients. The production of secondary metabolites (including lectins and toxic peptides) is of ultimate importance as a defence strategy in this context. The seedling is the most vulnerable lifestage inthe plants' life cycle, therefore it is not surprising that many species are well equipped with anti-nutritional factors andother substances in their seeds. Plants are often very economic in that they are able to utilize defence compounds (especially the N containing ones) as nitrogen source during germination. These compounds can be either acutely toxic (such as some lectins, cyanogenic glycosides, NPAAs or alkaloids), unpalatable (such as saponins, tannins, NPAAs, or bitteralkaloids) or "anti-nutritive" reducing growth and fitness of the consumer by nutrient complexation (e.g. by phytates), metabolic inhibition (e.g. NPAAs, cyanogenic glycosides, isoflavones, alkaloids) or reduction of digestion (e.g. through protease inhibitors, lectins, or oligosaccharides). Considering the evolutionary background of ANF as either anti herbivore or antimicrobial compounds, ANFs have been selected during evolution as biologically active components. If isolated and processed they could be even useful in medicine or in agriculture (increasing resistance against pests and pathogens; as natural plant protectants "biorational pesticide" or phytomedicines). The biological background and properties of legume ANFs is briefly summarized in the following.

Lectins and protease inhibitors

Those legumes which do not accumulate low molecular weight toxins in their seeds, often store toxic peptides (e.g. protease inhibitors), or lectins. Protease inhibitors block the function of digestive enzymes (proteases) in animals (leading to malnutrition and other disturbances), lectins binds to receptors in the intestinal tract and inhibit protein biosynthesis (e.g. the lectin abrine from the legume *Abrus precatorius* is one of the most potent toxins known).

Saponins

Triterpene saponins have been detected in soybean, lupins and several other legumes. Saponins are amphiphilous compounds which interact with biomembranes of animals, fungi and even bacteria. The hydrophobic part of the molecule complex cholesterol inside the membrane and their hydrophilic sugar side chain bind to external membrane proteins. Thus, fluidity of biomembranes is disturbed leading to holes and pores. As a consequence, cells become leaky and die. Legume saponins have only moderate toxicity and present a problem only when present in the diet at higher concentrations.

Phytate

Inositols with 4, 5 or 6 phosphate groups are common in the seeds of many of our grain legumes and can reach concentrations higher than 10% of dry matter. They can be regarded as stores for phosphate and mineral nutrients that are important for plant nutrition and especially valuable during germination. Since phytates complex iron, zinc, magnesium and calcium ions in the digestive tract, they can cause mineral ions deficiency in animals and humans.

Oligosaccharides and isoflavonoids

Legume seeds are generally rich in oligosaccharides (up to 20%), such as stachyose and raffinose. In animals, they produce flatulence and other disturbances. These compounds serve as carbon sources during germination; therefore their contents can be reduced in legumes through germination which is a common practice, e.g. in soybeans. Isoflavonoids have been detected in soybean, 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 interactions and exhibit estrogenic activities. Recently, it was found that the isoflavonegeniste in inhibits tyrosine kinases. 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.

Cyanogenic glycosides

Cyanogens are glycosides of 2-hydroxynitriles and widely distributed in Leguminosae (e.g. in *Phaseolus lunatus* and *Vicia sativa*). When plants are wounded by organisms, the cellular compart mentation breaks down and cyanogenic glycosides come into contact with an active ßglucosidase, which hydrolyses them to yield 2-hydroxynitrile. This is further cleaved into the corresponding aldehyde orketone and HCN by hydroxyl nitrilelyase. HCN is highly toxic for animals or microorganisms because it inhibits enzymes of the mitochondrial respiratory chain (cytochrome oxidases).

Pyrimidine glycosides

Vicine and convicine are ß-glycosides of the pyrimidines divicine and isouramil. High levels of vicine are present in the seeds of *V. sativa* and *V. faba* (which also contains significant levels of convicine). The ingestion of meals prepared from the seeds of *V. faba* can trigger the onset of Favism, an acute haemolytic disease which affects individuals lacking sufficient activity of the NADPH producing enzyme glucose-6-P-dehydrogenase (G6PD) in their red blood cells. Vicine and convicine have been implicated in favism because their hydrolysis products are unstable and formradicals which can cause a depletion of reduced glutathione (GSH) in G6PD deficient red blood cells.

Lupin alkaloids

Some species (*L. albus, L. luteus, L. angustifolius* and *L. mutabilis*) have big protein rich seeds (up to 50% protein), these lupins are of considerable agricultural interest. The main secondary metabolites of lupins are quinolizidine alkaloids which are sometimes accompanied by piperidine alkaloids, such as ammodendrine or indole alkaloids such as gramine. After synthesis in lupin leaf chloroplasts alkaloids are exported via the phloem all over the plant and are accumulated in the epidermis of leaves and stems. Reproductive organs, such as flowers and seeds are especially rich in alkaloids (2-6% dry weight). In germinating lupins alkaloids serve as a N source. Lupin alkaloids were shown to be feeding deterrents and lethal for a number of insects, especially aphids , but also moth- and butterfly larvae, beetles, grasshoppers, flies, bees and ants, other invertebrates and vertebrates, *i.e.* alkaloids are active over a wide range of animal orders .

Non-protein amino acids

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). Concentrations in seeds canexceed 10% of dry weight and since non-protein amino acids are remobilised during germination they certainly functionas N-storage compound. If non-protein amino acids are taken up by herbivores, microorganisms or other plants, they may interfere with several targets. They can be accepted in ribosomal protein biosynthesis in place of the normal amino acid leading to defective proteins (example: canavanine, azetidine-2-carboxylic acid). They can inhibit the charging of aminoacyl-tRNA synthetases or other steps of protein biosynthesis or they may competitively inhibit uptakesystems for amino acids. In vertebrates, effects may be among others: foetal malformations, neurotoxic disturbances, hallucinogenic effects, hair loss, diarrhoea, paralysis, liver cirrhosis, hypoglycemia, and arrhythmia. In plants, microorganisms and insects non-protein amino acids cause reduced growth or even death.

Elimination strategies

1. Post-harvest processing

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 the cooking water. All these simple techniques help to make legume seeds more palatable and digestible. Today, a deeper knowledge of the chemical structure of the anti-nutrients involved can help to devise technological strategies to process legume seeds in order to obtain toxin free products. Since a diversification will increase the economic value of the overall crop plant, food technology and rational processing are an alternative to breeding of ANF free plants which can be more susceptible to pests and pathogens. Followed by modern food technology (separation, filtration etc), pure nutritionally valuable dietary products, such as protein, dietary fibres, 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. Because of higher costs these considerations are related to the use of legume seeds which will be utilized for human consumption. For this purpose we need to develop varieties which are low in heat stable nutritionalfactors (e.g. alkaloids, saponins, phytates, isoflavones, 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 a widely known group like the temperate grain legumes.

2. Chemical detoxification

Deaminocanavanine is a well known untoxic deamination product of canavanine. The degradation of canavanine to deaminocanavanine under alkaline conditions provides 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 *Canavalia ensiformis*.

3. Fermentation

The use of fermentation as an integral part of food detoxification processes is widely practised. 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 preparedat both, an industrial and the household scale. The incorporation of fermentation processes into other simple food technologies also offers good prospects for a detoxification of food sources while simultaneously giving flexibility in the manipulation of flavour, texture and colour of the raw material.

4. 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 of such sproutsis excessive. The concentration of this compound 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 historyin Asia, where it has served to improve the palatability of soybeans.

5. Detoxification by ruminants

Vicia and *Lathyrus* grain legumes, *viz. V. faba*, *V. articulata, V. ervilia, V. sativa* and *V. narbonensis, L. sativus L. cicera, L. ochrus, L. clymenum* can be used as supplemental feeds for ruminant production, which is another form of post-harvest detoxification. Traditional practices are rapidly disappearing with the introduction of more profitable crops, while traditional crops often remain neglected by plant breeders. It is therefore important to preserve this knowledge and transfer it combined with plant introduction to regions where such crops can be grown with profit for ruminant production.

6. Can "anti-nutrients" be even useful?

There can be no doubt that the so called "anti-nutrients" of legumes have a biological function. They are certainly important in the physiology of seedlings as N or C storage compounds and to facilitate nutrient uptake and rhizosphere establishment. Since they are also toxic to animals and sometimes even to microorganisms, viruses and other plants, they exhibit defence functions at the same time. As shown for lupin alkaloids, ANFs can be important for the fitness of plants and constitute relevant resistance factors. "The development of a toxin-free crop would be totally impractical if the reduction in toxicity to man or domestic animals was accompanied by an equal or greater reduction in toxicity to predatory insects which might destroy the crop before it could be harvested". Lupin alkaloids, such as sparteine, have been used as antiarrhythmic and uterotonic therapeutics. Also other lupin alkaloids exhibit similar activities which could be used either as pure compounds or in mixtures. Isoflavonoids which inhibit tyrosine protein kinases might be interesting as anticancer agents.

7. Elimination of ANFs through genetic modification

The identification of alkaloids and NPAAs as the major anti-nutritional principles present in *Lupinus* and *Vicia* seeds, respectively. Now allows for the selection of genotypes with low levels of these factors, thus enabling the development of more palatable and less toxic cultivars. The selection of genetic material with contrasting levels of antinutritional factors is also ideally suited for the elucidation of their biological functions. The general aim is a selection of non-toxic and palatable genotypes requiring efficient screening techniques to expedite the quantitative detection of individual ANFs for the selection of improved grain legume cultivars. Such techniques should be suitable for testing large numbers of samples to facilitate the screening of the available germplasm and material generated through breeding or artificial mutagenesis. Sometimes simple colour reagents might work for an initial test, such as Reifers reagent for quinolizidine alkaloids in lupins. Immunological methods such as ELISA to detect specific proteins can also be established for low molecular weight compounds such as alkaloids. Whereas alkaloid rich bitter lupins are avoided by rabbits and aphids, sweet alkaloid poor varieties are readily accepted. A careful selection of plants attacked by generalist herbivores might provide a clue to plants with lower ANF levels.

8. Genetic engineering options

If the gene is known which encodes a toxic protein (e.g. a lectin) or the key enzyme of a biosynthetic pathway leading to alkaloids, saponins, NPAAs etc, genetic engineering offers a set of methodologies at present to down regulate or to knock out the respective activity. Strategies include the expression of antisense mRNA, of gene targetting, and of synthetic oligonucleotides or ribozymes. Also the introduction of new traits into a crop plant, such as new lectins or proteins rich in methionine/cysteinecan is achieved by appropriate molecular techniques. If a time-, developmental and organ specific expression is required then promoter sequences need to be known for aparticular plant and again these data are usually not available for the crop of interest. Transformed plants need to be regenerated which is a severe problem in most legumes.

Several target enzymes could be envisaged for NPAAs or alkaloids, but since the genes are still unknown, we have to leave this issue for a later conference.

Conclusion

Generally, the beneficial effect of legumes on human health is very important when consumed in significant amounts. ANCs are present in the legume and bean crops. The main adverse effects of the non-nutrient bioactive substances of pulses that could cause problems in human nutrition are antinutritional effects characteristic of insufficiently denatured enzyme inhibitors and lectin. A number of treatments of grain legumes are able to eliminate some bioactive substances partially or totally, including soaking, dry and moist heat treatment, filtration, germination, fermentation and enzymatic treatments. Obviously, chemical and physical characteristics determine the choice of appropriate treatment used to eliminate an undesirable compound from food. Therefore, it is probably not suitable to remove all these substances systematically by technological and especially by plant breeding means.

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