ANTINUTRITIONAL FACTORS IN FOOD LEGUMES AND EFFECTS OF PROCESSING

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Keywords: Food, legumes, antinutrients, toxins, processing, detoxification, antinutritional effects, beneficial effects, protease inhibitors, phytic acid, saponins, lectins, alpha-amylase inhibitors, β–ODAP, BOAA, α–galactosides

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Summary

Plants commonly synthesize a range of secondary metabolites as part of their protection against attack by herbivores, insects, and pathogens or as a 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 terms *antinutrient* or *natural toxicant* have been widely employed to describe plant defense metabolites in the food and nutrition literature. The observed biological effects vary greatly, depending upon the structures of the individual compounds, which can range from high molecular-weight proteins to simple amino acids and oligosaccharides.

Legumes are a rich source of antinutrients in the human dit. This article will focus on phytic acid, saponins, polyphenols, lathyrogens, α-galactosides, protease inhibitors, α-amylase inhibitors, and lectins which are found throughout grain and forage legumes.

The diverse structures of these compounds and methods for their analysis are discussed, the levels in raw and processed foods presented, and the natures of their biological effects described. A number of so-called antinutrients have been shown to possess beneficial—anticancer, antimicrobial—properties which also vary according to their level in the diet.

Brief reference to such effects are also included because a pressing challenge to scientists is to identify optimal levels for maximizing the benefit-to-risk ratio, thereby providing plant breeders, molecular biologists, and food processors with additional goals for maximizing overall product quality.

Methods for reducing antinutrients in food are presented according to their physical (dehulling/cooking; autoclaving/pressure cooking; dry roasting; soaking; milling/ ultrafiltration) or biochemical (enzyme processing; fermentation; germination) character. Given the importance of antinutrients to the developing plant, the complete removal of these compounds by breeding or biotechnological programs, even if possible, may produce plants with poorer growth and lower yield characteristics.

1. 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 defense against herbivores, insects, pathogens, or adverse growing conditions (Table 1). Many of these compounds may be labeled as antinutrients in the human diet due to their occurrence in fresh foods and processed foodstuffs.

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The structures of the antinutrients and their chemical properties, especially their heat-lability, dictate which physical processes will be most effective in their reduction or removal, thereby minimizing adverse biological effects.

It should be emphasized, however, that the complete removal of these antinutrients by breeding and biotechnological methods, even if this were possible, would result in the plants being characterized by poor growth or lower yields.

Low molecular weight ANF	Protein ANF and toxins
• β-ODAP* (nonprotein amino acids), Lathyrogens	• Allergens
• Cyanogens	• α-Amylase inhibitors*
• Flatulence factors (α-galactosides, oligosaccharids, e.g., raffinose)	• Lectins*
• Glucosinolates	• Lipase inhibitors
• Mycotoxins	• Protease inhibitors*
• Phytic acid*	• Transmissible spongiform encelopathies (TSE)**
• Saponins*	• Toxins (bacterial, marine, mushroom toxin)
• Polyphenols & tannins*	• Viruses*
• Others: alkaloids, gossypol, oestrogenic factors, sinapins	

* Antinutrients discussed in this article
** Largely unimportant for plant foods

Table 1. Some natural antinutritional factors and toxins in foods

Discussion of antinutrients or toxicants in foodstuffs must be put into context. With some notable exceptions, for example, the neurotoxin, β-ODAP, the reported deleterious effects of antinutritional factors are usually based upon observations and experimentation with rats, farming livestock, or other domestic animals.

As such, studies of oral toxicity involve feeding of relatively large amounts of feedstuff to animals over a relatively long time and as part of a relatively unvarying diet. In contrast, human consumption is frequently varied in terms of composition and time and increasingly involves highly processed food.

Undue reliance on individual plants or foods always carries with it the possibility of undesirable consequences, ranging from α-galactoside-derived flatulence through phytate-induced iron-deficiency (anemia] to, at its most serious, paralysis and death as associated with consumption of β-ODAP.

Plant food also contain proteinaceous antinutrients including protease inhibitors, phyto-haemagglutinins, or lectins, α-amylase inhibitors, and allergens. Consumption of raw

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legumes leads to nausea, vomiting, and diarrhea. More chronic effects of protein antinutrients include pancreatitis and some forms intestinal cancer although such effects have not been proven unequivocally.

A number of antinutrients have been shown to possess beneficial properties, for example, anticancer, antimicrobial). Such compounds are of increasing interest in the fields of biochemistry, medicine, pharmacology, and nutrition.

Plant breeders or molecular biologists wish to manipulate levels of these compounds to obtain optimal effect during cultivation while ensuring the quality of the harvested crop. In addition, the balance of adverse and beneficial properties referred to above should be borne in mind when breeding or molecular biological strategies are planned.

In this article, some diverse aspects of antinutrients are discussed within the general context of grain legumes; in this way comparative effects and trends may be more easily highlighted. The review also emphasizes the effect of common food processing techniques on legume antinutrients (Table 2). Legumes commonly used as foods include soybean (Glycine max), black gram (*Phaseolus mungo*), cowpea (*Vigna unguiculata*), dry bean (*Phaseolus vulgaris*), winged bean (*Psophocarpus tetragonolobus*), chickpea (*Cicer arietinum* L.), horse gram (*Dolichos biflorus*), moth bean (*Vigna aconitifolia*), pigeon pea (*Cajanus cajan*), fava bean (*Vicia faba* L. minor), grain amaranth (*Amaranthus* spp.), lentil (*Lens culinaris Medik*), jack bean (*Canavalia ensiformis*), sword bean (*Canavalia gliadata*), and grass pea (*Lathyrus sativus*). Although the total area of cultivation is believed to be similar for cereals and legumes, the latter contain a higher percentage of protein. Legume seeds provide a relatively affordable protein source in developing countries.

Physical and chemical methods employed to reduce or remove antinutritional factors include soaking, cooking, germination, fermentation, selective extraction, irradiation, and enzymic treatment.

The application of a single technique is frequently insufficient for effective treatment and so combinations are commonly employed; thus the most effective methods for reducing saponin contents have been reported to be soaking and cooking. Using a combination of abrasion and soaking, the amount of quinoa saponins was reduced by up to 100%.

Industrial processes, including canning, toasting, fractionation, and isolation of protein concentrates have also been shown to be effective in reducing or removing antinutritional factors. However, it should be borne in mind that processing can also introduce undesirable compounds, for example volatile aldehydes and ketones and peroxides, as a direct result of lipid oxidation, or reduce levels of desirable compounds, e.g., protein and essential minerals.

Only when attention is successfully given to removing antinutrients from foodstuffs can the full potential of those foods be realized; this is particularly true of foods (legumes being a prime example] from developing regions where the consequences of antinutrients may exacerbate malnutrition and disease.

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Physical Processing	Comments
Autoclaving, pressure cooking, steaming	Heating at ultrahigh temperatures (>100 °C). Performance dependent on temperature, moisture, pressure relations
Blanching	Mild boiling (75 °C – 95 °C) to inactivate endogenous enzymes and avoid cooking
Ordinary cooking	Usually preceded by soaking or another domestic processing, de-hulling, germination fermentation, and so on
Extrusion	A form of high temperature short time (HTST) processing involving a combination of high temperature, pressure, and shear processing
Roasting	Dry heating at 120 °C – 250 °C
Soaking	Exposure to water and salt solutions with or without additive to encourage ANF loss
Processing chemical and chemical modification	Treatment with thiols, sulphite, Cu-salts (± ascorbic acid). Chemical modification via acylation, succinylation

Table 2. Some physical processes for removing antinutrients from legume seeds

2. Phytic Acid, Saponins, Polyphenols, Lathyrogens, α-galactosides

2.2. Properties and General Characteristics

Phytic acid (PA). Myoinositol 1,2,3,4,5,6-hexakisdihydrogen phosphate (Figure 1) is found in cereals and legumes at levels ranging from 0.4% to 6.4%, by weight. PA is the primary phosphate reserve in most seeds accounting for 60% to 90% of total phosphorus. In monocotyledons such as wheat and rice, PA is present in the germ of corn and in the aleurone or bran layer allowing an easy separation by milling.

However, in dicotyledonous seeds such as legumes, nuts, and oilseeds, PA is found closely associated with proteins and is often isolated or concentrated with protein fraction of these foods.

Saponins. These are a diverse class of glycosides found mainly, but not exclusively, in plants; they comprise a steroidal or triterpene aglycone linked to one, two, or three saccharide chains of varying size and complexity via ester and/or ether linkages (Figure 1). The most common sugars linked to an aglycone [or sapgenin] are galactose, arabinose, xylose and glucose.

The amphiphilic behavior of a saponin is a result of the opposing lyophilic and lyophobic characteristics of the carbohydrate and aglycone moieties; thus the structure affects its chemical behavior and leads to the expression of many of its biological properties. Changes in saponin structure, perhaps as a result of partial hydrolysis during processing, will therefore have significant effects on the quality of the processed product.

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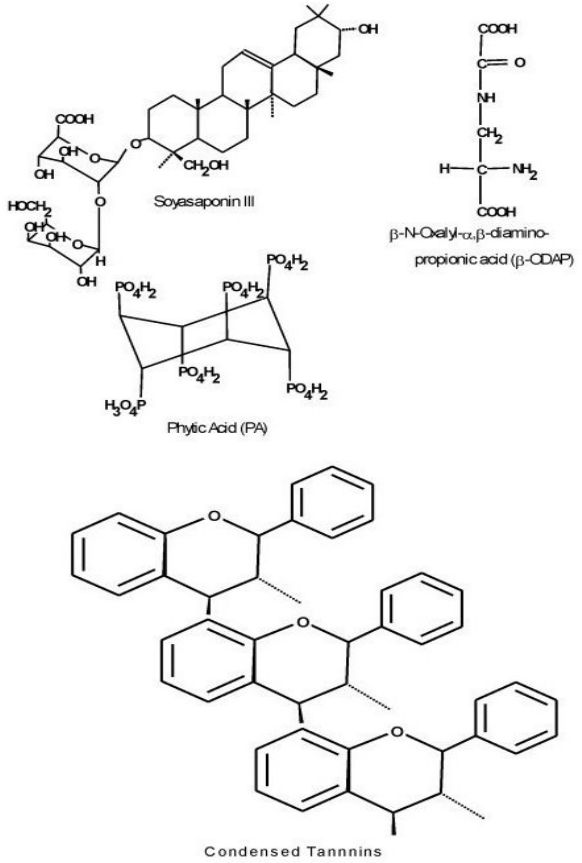

Figure 1. The structure of some low molecular weight antinutrients

Saponins occur in a broad range of plants consumed in the human diet, including legumes (soya, peas, and beans), root crops (potato, yams, asparagus, and alliums) as well as in oats, sugar beet, tea, and many medicinal herbs (such as ginseng). Within grain legumes, the saponin content varies between 0.5% and 5% dry weight, with soybean being the most important dietary source (Table 3).

Source	Saponins (% dry weight)
Soybeans (*Glycine max*)	5.6
Chickpea *(Cicer arietinum* L.)	3.6
Lucerne (*Medicago sativa)*	2.5
Lupine (*Lupinus angustifolius*)	1.5
Quinoa (*Chenopodium quinoa*) Wild)	0.02–0.04 (0.47–1.13)*
Ginseng	0.5–3.0

* values for sweet and bitter varieties

Table 3. Saponin levels in some legume seeds and ginseng

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Saponins, as a family, are generally characterized by their bitter taste (those in liquorice are an exception, being sweet], their ability to foam in aqueous solutions, and their ability to hemolyse red blood cells, these latter properties being a consequence of their amphiphilic properties. Ingestion of saponin-containing foods by man and animals has been associated with both deleterious and beneficial effects; for example, reduced weight in animals and hypochlesterolemic effects in man. Many of the properties can be attributed to the ability of saponins to affect membrane integrity.

Following oral administration, saponins are only poorly absorbed and are either excreted unchanged or metabolized in the gut. Detailed information on the fate of saponins in the animal gut is lacking, but enteric bacteria, intestinal enzymes, and/or gastric juices most likely cause breakdown within the gastrointestinal tract. The amphiphilic character of saponins makes them natural surfactants and they interact readily with cells, the aglycone moiety binding to the lipophyllic membrane. Quinoa saponins have been reported to be membranolytic against cells of the small intestine and to cause an increase in mucosal permeability *in vitro*.

Polyphenols. These secondary plant metabolites are distributed ubiquitously within plant foods (vegetables, cereals, legumes, fruits, nuts, and so on) and beverages (tea, wine, cocoa, and so on). Their levels vary greatly even between cultivars of the same species. Environmental factors such as light, germination, degree of ripeness, variety, processing and storage, and genetic factors (see Table 4) can influence the levels. Currently there is no accurate information available on dietary intake of polyphenols; only a few estimates are available which are 1g/day (US), 23mg/day (Dutch), and 28 mg/day (Denmark).

Food	mg/100g dry matter
Cereals	22–102.60
Legumes	34–1710
Nuts	0.04–38
Vegetables	6–2025 (mg/fresh matter)
Fruits	2–1200 (mg/fresh matter)
Beverages	
Tea	150–210 (mg/ 200ml)
Red wine	1000–4000 (mg/L)
White wine	200–300 (mg/L)

Table 4. Polyphenolic content of different plant foods and beverages (adapted from Bravo, 1998)

Oxidation products of phenolic compounds are known to be involved in plant defense against insects, pests, and invading pathogens including bacteria, fungi, and viruses; polymeric compounds appear to be more toxic toward phytopathogens than their precursor low-molecular weight monomers, such as chlorogenic acid.

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Polyphenoloxidase-catalysed polymerization helps seal the injured plant surface and begin the healing process.

Enzyme-catalyzed browning reactions of polyphenols continue after the food is harvested resulting in deterioration of flavor, color, and nutritional quality. For this reason, prevention of enzymatic browning in fruits and vegetables has long been a major concern of food scientists.

Lathyrogens. β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP or BOAA), a naturally occurring amino acid (Figure 1), possesses potent neurotoxic activity and has been shown to be responsible for outbreaks of neurolathyrism following consumption of *Lathyrus sativus***.** β-ODAP occurs naturally as two isomeric forms with the α-form being approximately 5% of the total. According to toxicological studies, this isomer is less toxic than the major, β-isomer. The level of β-ODAP in dry seed varies considerably according to genetic factors and environmental conditions. *L. sativus* grown in nutrient solutions that are zinc-deficient or rich in ferrous iron produced seed with elevated levels of β-ODAP. Levels of this neurotoxin varied between 1.25g/kg and 25g/kg in seeds obtained from Ethiopia, with a narrower range of 5 g/kg to 8g/kg in seed from Bangladesh. β-ODAP is biosynthesized during the ripening of the seed and is further increased during germination.

α-Galactosides. Flatulence-causing oligosaccharides, chemically described as α-galactosides, are present in significant amounts in mature legume seeds.

2.2. Analyses

Phytic Acid. Extraction, by ion exchange chromatography, hydrolysis, and colorimetric determination of phosphorus is the most commonly used method of analysis. However, only certain inositol phosphate molecules are biologically active and so these methods will lead to overestimates; as a result there is increasing demand for methods capable of separating and measuring individual inositol phosphates (resulting from natural degradation by phosphatases and specific phytases] in foodstuffs. The introduction of such methods has been inhibited by the lack of a suitable UV-chromophore in this group of molecules. This has led to the use of low-sensitivity refractive index detectors, which prohibit use of gradient elution techniques. Capillary electrophoresis, combined with indirect detection using 1-naphthol-3, 6-disulphonic acid is reported to show promise. Using such a method, all six inositol phosphates have been separated and analyzed over a period of 13 minutes.

Saponins. While many methods of analysis have been proposed, there is no standard method which can be applied across the plant kingdom. The classical methods, for example, gravimetry or assaying haemolytic activity, are now of historic interest only. A great number of chromatographic methods have been employed, with HPLC being common, albeit time-consuming. Of particular importance is the fact that variation in aglycone structure, and in the nature and linkages of the carbohydrate moieties, may have significant effects on biological activity, and hence total saponin figures are generally of little usefulness. The chemical composition of saponins may be determined by acid hydrolysis and separation of the carbohydrate and aglycone moieties; GC and/or

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proton or carbon-13 NMR may determine the latter. The integration of silica gel column chromatography, semi-preparative HPLC, and repeated preparative TLC may afford the desired separation in most cases. The problem with most studies is that it is impossible to determine whether the observed losses of saponins are due to thermally induced degradation or is a result of the formation of poorly extractable saponin complexes.

Polyphenols and Tannins. Colorimetric methods, for example, vanillin-HCl, Folin-Ciocalteu, are still commonly used, as is the FRAP method, based upon reduction of ferric ion to ferrous by tannins, but HPLC, employing gradient or isocratic techniques, of polyphenols is increasingly used for both groups and individual compounds.

β-ODAP. This is an amino acid and so its reaction with ninhydrin has been used for its detection and estimation. Alkaline hydrolysis converts both ODAP isomers to DAP and so colorimetric assay following alkaline treatment measures combined levels of both. Several attempts to develop a specific, ELISA, assay for the β-isomer have failed; the measurement of the β-isomer by an enzymatic flow injection technique that uses an immobilized glutamic oxidase has been achieved. One of the most commonly used methods involves extraction of both ODAP isomers followed by quantitative analysis, as DAP. An electrophoretic method for separating the two isomers, followed by individual colorimetric assay, has been developed.

α-Galactosides. A wide range of chromatographic methods has been employed, but HPLC is the method of choice. A silica-amino column is most commonly employed with acetonitrile-water mixture as mobile phase; variations described in the literature generally involve differences in extraction and cleanup procedures. Determination of the free sugar digestibility index, defined as the ratio of digestible sugars to non-digestible sugars, can assist breeders' selection of plant material to ensure flatulence-producing, high α-galactoside lines are identified and eliminated.

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Biographical Sketches

Santosh Khokhar was appointed Senior Lecturer in Food Biochemistry and Nutrition at the Procter Department of Food Science in September 1998. Her main research interests centre on food composition and health with special emphasis on the diets of minority populations.

Following her graduation from Haryana Agricultural University, Santosh joined its Department of Foods and Nutrition as Assistant Professor. In 1993 she spent a year at the Institute of Food Research in Norwich as Marie Curie Postdoctoral Fellow. This was followed by research appointments in The Netherlands - at the Department of Food Science of the Agricultural University in Wageningen, the State Institute for Quality Control of Agricultural Products [RIKILT-DLO] and the TNO-voeding, Zeist (Institute of Applied Research in Food and Nutrition).

Richard Owusu-Apenten joined Department of Food Science, Pennsylvania State University in August 2001 as an Associate Professor in Food Science with emphasize on Food Proteins. His appointment is 60% research and 40% teaching. Dr Owusu-Apenten obtained his BSc (Honors) degree in biochemistry from the University of London (England). He has a PhD degree in chemistry from the same university. His past research work included, immobilized enzymes, ion selective electrodes for plant herbicides, enzyme production by nonpathogenic microorganisms from extreme environments, and food protein ingredients. Richard worked as a faculty member at the Department of Food Science (University of Leeds) for 12 years prior to joining the faculty at Pennsylvania State University.

Dr Owusu-Apenten's current research interests are –the effect of protein stability on applications in the food system. Topics of interest includes protein ingredient functionality and the role of proteins in nutrition and food safety. Richard's teaching interests cover general food science, nutrition and applied protein chemistry. He has written one book (Food Proteins: Quantitative analysis and the effect on processing) and is currently working on his second.

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