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Role of Anti-nutritional Factors in Food Industry

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ABSTRACT

Anti-nutritional factors reduce the nutrient utilization and/or food intake of plants or plant products used as human foods or animal feeds and they play a vital role in determining the use of plants for humans and animals. Apart from cyanogenic glycosides, food poisoning arising from anti-nutritional factors, otherwise known as plants' secondary metabolites has not been properly addressed in most parts of the developing world. People have died out of ignorance, poverty and inadequate nutrition information and education, especially within the African societies. There are reports from time to time of deaths after consumption of some type of beans despite cooking. Also, cases of renal and liver diseases are increasing and this call for a need to properly address the issue of thorough and adequate processing of foods/feeds before consumption. The aim of this review is to emphasize on the adequate processing of foods/feeds and to educate the people on the dangers of consuming improperly processed foods especially legumes which are reported to contain very high concentrations of anti-nutritional factors. There is a wide distribution of biologically-active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition. The knowledge that these compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems. It is well known that plants generally contain anti-nutrients acquired from fertilizer and pesticides and several naturally-occurring chemicals. Some of these chemicals are known as "secondary metabolites" and they have been shown to be highly biologically active. They include saponins, tannins, flavonoids, alkaloids, trypsin (protease) inhibitors, oxalates, phytates, haemagglutinins (lectins), cyanogenic glycosides, cardiac glycosides, coumarins and gossypol. The list is inexhaustible. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed in appropriate amounts after adequate processing.

INTRODUCTION

Most of these secondary metabolites elicit very harmful biological responses, while some are widely applied in nutrition and as pharmacologically-active agents. The pharmacological and other beneficial effects of these anti-nutritional factors in plants have been reviewed by researchers. Food poisoning arising from plant secondary metabolites, otherwise known as anti-nutritional factors, other than cyanogenic glycosides, has not been properly addressed in Nigeria, and indeed in most parts of the developing world/countries, that depend/feed mostly on vegetable-based diets. People have therefore died out of ignorance, poverty and inadequate nutrition education, especially within the African societies. The anti-nutritional factors (ANFS) may be defined as those substances generated in

natural food stuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition. Being an ANF is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. For example, trypsin inhibitors, which are ANFs for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen. The utility of the leaves, pods and edible twigs of shrubs and trees as animal feed is limited by the presence of ANFs. Much of the available data and information on the nutrient and anti-nutrient composition of the more commonly used local foods and feeds do not cover all the foods and feeds and where available, needs updating. This is because of the possible effects of variety/genetic origin, climate, soil, processing methods, pesticides and fertilizers on the chemical composition of the food plants. Several considerations justify the continued surveillance, knowledge and future research on anti-nutritional factors/toxic substances naturally occurring in plants used as foods and feedstuffs and ways of reducing them to safe level of consumption.

Firstly, the possibility that newly developed or exotic foods may contain natural toxicants must be taken into account in evaluating their usefulness (Osagie, 1998). Also, introduction of new plant varieties into our diets may expose humans and animals to new toxic factors with unsuspected biological effects.

Secondly, improper processing of plant food like beans and pulses may expose humans and animals to high concentrations of these toxic factors. An example is the production of soymilk and its use as an alternative to cow's milk in infant formula. It is reported that soymilk, if not properly processed and supplemented with Iodine, causes goiter in infants. Raw soybeans also cause goiter in rats and chicks.

Thirdly, these anti-nutritional factors are increasingly recognized as significant items of the diet of humans and animals. Thus, they affect the overall nutritional value of foods and feeds.

Fourthly, the plant breeder, in an attempt to develop higher-yielding or disease-resistant crop varieties must, at the same time, be alert to the possible production of undesirable components.

Fifthly, amounts of certain food substances that are relatively safe when consumed individually can sometimes, when taken together, have serious and even fatal effects. An example is the presence of tannins in a protein-marginal diet.

Sixthly, Public health authorities and other food regulatory bodies need to be informed about the possible dangers related to the widespread, longstanding practices previously regarded as safe. For example, cassava and some legumes have been consumed in the tropics for a long time without apparent consideration for their high cyanide contents. Also, information on the anti-nutritional components of foods/feedstuffs will help dieticians, veterinarians, human and

animal nutritionists to avoid recommending foods/feeds that their patients cannot tolerate, possibly because of the inability to metabolize or detoxify certain substances there in. In the last few decades or more, large numbers of scientific data have emerged, linking diet and food selection patterns to the maintenance of health and the prevention of some chronic diseases. There is a consensus among physicians that nutrition constitutes an essential aspect of health care.

ANTI-NUTRITIONAL FACTORS (PLANTS' SECONDARY METABOLITES)

The abundance of anti-nutritional factors and toxic substances in plants used as human foods and animal feeds certainly calls for concern. Therefore, ways and means of eliminating or reducing their levels to the barest minimum should be discovered. It could be wrongly argued that since the African cultural method of preparing food involves cooking, there is no cause for alarm. This is not entirely correct because although the toxic effects of most anti-nutritional factors present in plant food and feedstuffs can generally be eliminated by proper heat treatment, it should be appreciated that conditions may prevail whereby complete destruction may not always be achieved. For example, it has been reported that phytic acids (phytates) are not destroyed by being cooked even in boiling water. The mixtures of ground beans and ground cereals prepared under the field conditions prevailing in Africa, the haemagglutinins not always completely destroyed, and that cooked products produced diarrhea and other toxicity signs. A reduction in the boiling point of water in mountainous regions could also result in incomplete destruction of toxicity or incomplete elimination of the lectins. Also, people do not patiently prepare or process beans to the recommended level to destroy the anti-nutrients because of the high and prohibitive cost of energy sources like kerosene and gas and the scarcity of firewood, especially during the rainy season. As a result of this, people are forced to eat improperly cooked nutritionally toxic beans. There has also been some press reports of food poisoning leading to mortality in humans after consumption of bean meal. The adverse effect of excessive cooking on protein denaturation is also an important factor to be considered during food / feed processing.

A review of the occurrence of nitrate in unprocessed foods showed that high concentrations are frequently found in vegetables. Nitrates are present in all plants and are an essential source of nitrogen for normal growth. The increasing incidence of various forms of cancer in the world at large and in Nigeria in particular may be attributed to the levels of certain chemicals in our foods and drinks.

The consequences/implications of toxic factors in plants used as food/feedstuffs are not their direct toxicity to man and animals alone, but also the inconvenience and the economic loss associated with poisoning of domestic animals and the cost of preventing or reducing such happenings. It is worthy of note that plant poisons can either be accumulated in the animal or in certain organs or they are metabolized and excreted in milk. Ruminants may convert cyanide to the less toxic thiocyanate, which is goitrogenic. By this food chain, toxins or their metabolites thereof may become harmful to man. Nutrition education should emphasize adequate and thorough preparations of human foods and animal feeds, especially in humans where there are increases in reported cases of renal diseases. Oxalate, which is an anti-nutritional component of plant, if consumed in large quantities is associated with blockage of renal tubules by calcium oxalate crystals and development of urinary calculi. All these can lead to renal disease and hence death in susceptible individuals. There is also the need to re-visit studies on the levels of anti-nutritional factors in our common foods due to the influence of soil, fertilizer, pesticides, and other chemicals and environmental conditions on the levels of these anti-nutritional factors in plants.

Classification of Anti-Nutritional Factors

The anti-nutritional factors in plants may be classified on the basis of their chemical structure, the specific actions they bring about or their biosynthetic origin. Although this classification does not encompass all the known groups of anti-nutritional factors, it does

present the list of those frequently found in human foods and animal feedstuffs. The anti-nutritional factors may be divided into two major categories.

- (1) Proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures.
- (2) Other and which include, among many others, polyphenolic compounds (mainly condensed tannins), non-protein amino acids and galactomannan gums. More often than not, a single plant may contain two or more toxic compounds, generally drawn from the two categories, which add to the difficulties of detoxification. There are several anti nutritional factors that are very significant in plants used for human foods and animal feeds. They are
 - (i) Enzyme inhibitors (trypsin and chymotrypsin inhibitors, plasmin inhibitors, elastase inhibitors),
 - (ii) Haemagglutinins (concanavalin A, ricin),
 - (iii) Plant enzymes (urease, lipoxygenase),
 - (iv) Cyanogenic glycosides (phaseolunatin, dhurrin, linamarin, luteostralin),
 - (v) Goitrogens (pro-goitrins and glucosinolates),
 - (vi) Oestrogens (flavones and genistein),
 - (vii) Saponins (soya sapogenin),
 - (viii) Gossypol from *Gossypium species* e.g. cotton,
 - (ix) Tannins (condensed and hydrolysable tannins),
 - (x) Amino acid analogues (BOAA, DAP, mimosine, N-methyl-1-alanine),
 - (xi) Alkaloids (solanine and chaconine),
 - (xii) Anti-metals (phytates and oxalates),
 - (xiii) Anti-vitamins (anti-vitamins A, D, E and B12) and
 - (xiv) Favism factors.

Mimosine

Mimosine, a non-protein amino acid structurally similar to tyrosine, occurs in a few species of *Mimosa* and all species of closely allied genus *Leucaena*. Concern has arisen because of the importance of *L. leucocephala*, in which the level of Mimosine in the leaf is about 2-6% and varies with seasons and maturity. In non-ruminant animals, Mimosine causes poor growth, alopecia, eye cataracts and reproductive problems. Levels of *Leucaena* meal above 5-10% of the diet for swine, poultry and rabbits generally result in poor animal performance. The mechanism of action of Mimosine in producing its effect is not clear but it may act as an amino acid antagonist or may complex with pyridoxal phosphate, leading to disruption of catalytic action of B6-containing enzymes such as trans-aminases, or may complex with metals such as zinc. The main symptoms of toxicity in ruminants are poor growth, loss of hair and wool, swollen and raw coronets above the hooves, lameness. mouth and Oesophageal lesions, depressed serum thyroxine level and Goitre. Some of these symptoms may be due to Mimosine and others to 3, 4 dihydroxypyridine, a metabolite of mimosine in the rumen. Toxic signs like skin lesions also resemble Zn deficiency. Reduction in calving percentage due to *Leucaena* feeding has also been noted. A solution to the mimosine problem could be the development of low Mimosine cultivars. However, low mimosine types are found to be unproductive and of low vigor. The other approach is to feed *Leucaena* mixed with other feeds. The use of *Leucaena* fodder may be restricted to 30 % of the green forage in the case of cattle and buffalo, and 50% for goats. The effect of *Leucaena* and mimosine can be reduced by heat treatment, by supplementation with amino acids or with metal ions such as Fe²⁺, and Zn²⁺.

Polyphenols

Also known phenolic compounds, are group of chemical substances present in plants (fruits, vegetables). Phenolic compounds are responsible for the color of many plants, such as apples, they are part of the taste and flavor of beverages (apple juice, tea), and are important anti-oxidants in plants. Polyphenols are normally complex organic substances, which contain more than one phenol group. Polyphenols can be divided into many different sub categories, such as anthocyanins (colors in fruits), flavonoids (catechins, tannins in tea

and wine) and non-flavonoids components (gallic acid in tea leaves). Flavonoids are formed in plants from the aromatic amino acids phenylalanine and tyrosine.

Cyanogens

Cyanogens are glycosides of a sugar, or sugars, and cyanide containing a glycone. Cyanogens can be hydrolysed by enzymes to release HCN which is volatile gas. However, the glycosides occur in vacuoles in plant cell and enzymes are found in the cytosol. Damage to the plant results in the enzymes and glycoside coming together and producing HCN. The hydrolytic reaction can take place in the rumen by microbial activity. Hence ruminants are more susceptible to HCN toxicity than non-ruminants. The HCN is absorbed and is rapidly detoxified in the liver by the enzyme Rhodanese which converts HCN to thiocyanate (SCN). Excess cyanide ion inhibits the cytochrome oxidase. This stops ATP formation, tissues suffer energy deprivation and death follows rapidly. The lethal dose of HCN for cattle and sheep is 2.0-4.0mg per kg body weight. The lethal dose for cyanogens would be 10-20 times greater because the HCN comprises 5-10% of their molecular weight. For poisoning, forage containing this amount of cyanogens would have to be consumed within a few minutes and simultaneous HCN production would have to be rapid. Recorded accounts of livestock poisoning by cyanogenic plants show that such situations do arise. Cyanide can cause goitrogenic effects due to thiocyanate produced during detoxification. Poor animal performance due to *A. sieberiana* pod feeding has been attributed to cyanogens. Cyanogens have also been suspected to have teratogenic effects. Post-harvest wilting of cyanogenic leaves may reduce the risk of cyanide toxicity. Animals suffering from cyanide must be immediately treated by injecting suitable dose of sodium nitrate and sodium thiosulphate.

Saponins

Saponins are glycosides containing a polycyclic a glycone moiety of either C₂₇ steroid or C₃₀, triterpenoid (collectively termed as sapogenins) attached to a carbohydrate.

Saponins are characterized by a bitter taste and foaming properties. Erythrocytes lyse in saponin solution and so these compounds are toxic when injected intravenously. The anti-nutritional effects of saponins have been mainly studied using alfalfa saponins. In non-ruminants (chicks and pigs), retardation of growth rate, due primarily to reduction in feed intake, is probably the major concern. Such effects have also been noted when *Sesbania sesban* leaf meal (saponin 0.71 %) was incorporated in a chick diet. In ruminants, saponins were implicated in causing bloat. However, later studies indicate that they are not involved in the bloat syndrome. Furthermore, because saponins may also undergo bacterial degradation in the rumen, they may not retard the growth of ruminants. Nevertheless, recent studies indicate that they inhibit microbial fermentation and synthesis in the rumen.

In ruminants, some reports of toxicity due to dietary saponins have also appeared. It is observed that 4-7 weeks feeding of *Albizia stipulata* gave rise to toxic manifestations in sheep.

The toxicity of broom weed (*Gulierrezia sarothrae*), a resinous shrub, is believed to be due to its saponin content. Symptoms include listlessness, anorexia, weight loss and gastroenteritis. These results indicate that saponins from different plant species have varied biological effects probably due to structural differences in their sapogenin fractions. The adverse effects of saponins can be overcome by repeated washing with water which makes the feed more palatable by reducing the bitterness associated with saponins.

Phytohemagglutinins

Phytohemagglutinins, otherwise referred to as lectins, are proteins which agglutinate red blood cells. They have been shown to occur in some important fodder trees. The highest concentrations of lectins are found in seeds but, in the leaves, their concentration is low due to translocation. The biological effects of lectins probably result from their affinity for sugars. They may bind to the carbohydrate moieties of cells of the intestinal wall and cause a non-specific interference with nutrient absorption. In fodder trees, the lectins of interest are

robin and ricin. Robin, a lectin from *Robinia pseudoacacia*, has been reported to cause symptoms of anorexia, lassitude, weakness and posterior paralysis in cattle. Ricin occurs in castor beans (*Ricinus communis*) which have been reported to cause poisoning in all class of livestock. Due to ricin, deoiled castor seed cake (CP 35 %) is seldom used as a livestock feed. However, the mature leaves of *R. communis* have been found suitable for feeding to sheep; hence precautions against bean contamination are necessary. Castor bean meal can be detoxified by autoclaving at 20 psi for 60 minutes for incorporation in sheep diets.

Tannins

Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur almost in all vascular plants. Hydrolysable tannins and condensed tannins (proanthocyanidins) are two different groups of these compounds. Generally tree and shrub leaves contain both types of tannins. The two types differ in their nutritional and toxic effects. The condensed tannins have a more profound digestibility-reducing effect than hydrolysable tannins, whereas the latter may cause varied toxic manifestations due to hydrolysis in rumen. Sheep ingesting 0.9g hydrolysable tannins kg of body weight showed signs of toxicity in 15 days. Animals like mule, deer, rats and mice have been shown to secrete proline-rich proteins in saliva which constitute the first line of defence against ingested tannins. Nevertheless, deleterious effects and episodes of toxicity suggest the inadequacy of defense against high quantities of dietary tannins. The mechanism of dietary effects of tannins may be understood by their ability to form complex with proteins. Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes. Tannin-protein complexes involve both hydrogen-bonding and hydrophobic interactions; the precipitation of the protein-tannin complex depends upon pH, ionic strength and molecular size of tannins. Both the protein precipitation and incorporation of tannin phenolics into the precipitate increase with the increase in molecular size of tannins. However, when the molecular weight is very large (> 5000), the tannins become insoluble and lose their protein precipitating capacity. Hence the measurement of the phenolic profile in terms of total phenols, condensed tannins, their protein precipitating capacity and degree of polymerization becomes imperative to assess the role of tannins in ruminant nutrition. In tree leaves tannins are present in NDF and ADF in significant amounts which are tightly bound to the cell wall and cell protein and seem to be involved in decreasing digestibility. Hence, there is a need to account for these tannins in estimating the nutritive value of tree leaves. In ruminants, dietary condensed tannins (2-3%) have been shown to impart beneficial effects because they reduce the wasteful protein degradation in the rumen by the formation of a protein-tannin complex. The complex appears to dissociate post-ruminally at a low pH where, presumably, the protein becomes available for digestion. However, free condensed tannins would probably be available to form a complex with digestive enzymes such as pepsin and also with the protein of gut wall. Condensed tannins of *Prosopis cineraria* precipitate pepsin at pH 2.0 and the net effect of the presence of condensed tannins may therefore be negligible. Alkaloids such as N-methyl- α -phenethylamine cause locomotor ataxia of the hindquarters in sheep. Sesbaine causes haemorrhagic diarrhoea. The terpenoids azadirachtin and limonin impart a bitter taste and the leaves of *Azadirachta indica* are therefore not relished by cattle. Oxalate in the leaves of *Acacia aneura* may limit the Ca availability and a negative correlation between digestibility and lignin content in tropical browse has been observed. Anti-nutrients are chemical substances in food that do not offer nourishment to the body. E.g. phytic acid, oxalates, tannins and hydrocyanic acid. The effect of these anti-nutrients in the body depends on the type and the concentration in which it is present in the food material. Phytic acid lowers the utilization of elements like calcium, magnesium, zinc and iron due to its ability to form insoluble salts with their ions. The phytic

acid content of 64 foodstuffs and found that 20-60% of the phytates in cereals are excreted by human beings unchanged in the faeces. It has also been reported that the maximum tolerable dose of phytates in the body is from 250 to 500mg/100g. Oxalate is produced and accumulated in many crop plants and pasture weeds. When taken into the body, oxalates do not only combine with calcium but also with magnesium to form insoluble salts which are not available to the body. Cyanides are a group of O-glycosides formed from decarboxylated amino acids. Cyanides can inhibit cellular oxidation by combining with the catalytic ion of cytochrome oxidase leading to elimination of the active unit concerned with transfer of electrons to molecular oxygen. Hydrocyanic acid has also been found to inhibit the activity of vitamin K dependent carboxylase of the liver. Tannins are polyhydric phenols. The two distinctive groups are the hydrolysable tannins (so called because they may be readily hydrolyzed into a mixture of carbohydrates and phenols) and condensed tannins, which are complex flavonoids polymers. Tannins form insoluble complexes with proteins, carbohydrates and lipids leading to a reduction in digestibility of these nutrients. Other nutritional effects that have been attributed to tannins include damage to the intestinal tract, toxicity of tannins absorbed from the gut, interference with the absorption of iron and a possible carcinogenic effect.

Phytic Acid

Phytic acid forms insoluble complexes with calcium, zinc, iron and copper. Proteins can also be anti-nutrients, such as the trypsin inhibitors and lectins found in legumes. These enzyme inhibitors interfere with digestion. Another particularly widespread form of anti-nutrients are the flavonoids, which are a group of polyphenolic compounds that include tannins. These compounds chelate metals such as iron and zinc and reduce the absorption of these nutrients, but they also inhibit digestive enzymes and may also precipitate proteins. However, polyphenols such as tannins have anticancer properties, so drinks such as green tea that contain large amounts of these compounds might be good for the health of some people despite their anti-nutrient properties. Anti-nutrients are found at some level in almost all foods for a variety of reasons. However, their levels are reduced in modern crops, probably as an outcome of the process of domestication. Nevertheless, the large fraction of modern diets that come from a few crops, particularly cereals, has raised concerns about the effects of the anti-nutrients in these crops on human health. The possibility now exists to eliminate anti-nutrients entirely using genetic engineering; but, since these compounds may also have beneficial effects (such as polyphenols reduce the risk of cancer, heart disease or diabetes), such genetic modifications could make the foods more nutritious but not improve people's health. Many traditional methods of food preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods through reducing certain anti-nutrients such as Phytic acid, Polyphenols, and oxalic acid. Such processing methods are widely-used in societies where cereals and legumes form a major part of the diet. An important example of such processing is the fermentation of cassava to produce cassava flour: this fermentation reduces the levels of both toxins and anti-nutrients in the tuber.

Phytic acid is an anti-nutrient that interferes with the absorption of minerals from the diet. Phytic acid (known as inositol hexakisphosphate (IP6), or phytate or its salts) is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is not digestible to humans or no ruminant animals, however, so it is not a source of either inositol or phosphate if eaten directly. Moreover, it chelates and thus makes unabsorbable certain important minor minerals such as zinc and iron, and to a lesser extent, also macro-minerals such as calcium and magnesium. Catabolites of Phytic acid are called lower inositol polyphosphates.

Anti-Nutritional Factors in Soybean

Among the anti-nutritional factors present in soybean seed, the main ones are protease inhibitors—Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor, and lectins. Protease inhibitors represent 6%

of the protein present in soybean seed. Approximately, 80% of the trypsin inhibition is caused by KTI, which strongly inhibits trypsin and therefore reduces food intake by diminishing their digestion and absorption. Another effect of KTI is the induction of pancreatic enzyme, hyper secretion and the fast stimulation of pancreas growth, hypertrophy and hyperplasia. Due to this, raw soybean can not be used for feeding monogastric animals. Heat treatment doesn't completely eliminate these factors and may decrease protein solubility. Despite the efficiency of thermal treatment to reduce protease inhibitors, residual inhibition (10-20%) is maintained. By this reason, a part of the breeding program of the Maize Research Institute Zemun Polje is aimed at developing soybean cultivars with reduced trypsin inhibitors content. As a result, two cultivars lacking KTI, Lana and Laura, were released. The trypsin inhibitor content in these cultivars ranges from 15.01 mg g⁻¹ in Laura to 15.35 mg g⁻¹ in Lana, which is about 50% reduced as compared to the genotypes with standard grain type. The utilization of these cultivars is a great opportunity for saving energy and preserving valuable nutritional composition of soybean grain, which is of interest in industrial processing. This trait makes them also suitable for direct feeding in adult non-ruminant animals without previous thermal processing. Lectins are proteins that are widely distributed in plant kingdom and have unique property of binding carbohydrate-containing molecules with a high specificity, causing agglutination of red blood cells. Soybean agglutinin (SBA) causes the atrophy of the microvilli, reduces the viability of the epithelial cells and increases the weight of small intestine because of hyperplasia of crypt cells. The inactivation of soybean lectin by a moist heat treatment is parallel with the destruction of trypsin inhibitors. Soybean lectin is quite resistant to inactivation by dry heat treatment. The soybean genotypes lacking SBA were found. The comparison of relative contribution of KTI and SBA on chicken growth revealed a greater anti-nutritional effect of KTI than of SBA.

Soybean contain high amount of oligosaccharides, consisting mainly of raffinose and stachyose. These oligosaccharides are poorly digested and have been implicated as causes for the poor utilization of energy from soybean meal fed to poultry. Raffinose and stachyose are heat stable; the attempts have been made to eliminate them by enzymatic action and selecting desirable soybean varieties. Phytic acid is present in soybean seed and products to the extent of 1-1.5% of DM. It is able to chelate mineral elements, such as zinc, magnesium, iron, calcium and potassium and makes these elements longer absorbed from intestines. About two thirds of the total phosphorus from soybean seed is bound to phytic acid. Several soybean genotypes have been developed with a low phytic acid content, often featured with lower grain yield and seed viability. More breeding cycles are needed to improve a cultivar performance and keep phytic acid at a low level.

Beside mentioned anti-nutritional factors, soybean contains physiologically active compounds with small or unknown effects, such as tannins, saponins, antivitamins and isoflavones.

Anti-Nutritional Factors in Feed Pea

Main anti-nutritional factors in pea are trypsin inhibitors and lectins. As in other grain legumes, these factors have a proteinaceous nature, belonging to albumins, and are inactivated by high temperatures or soaking in formaldehyde. According to their trypsin inhibitor activity, expressed by trypsin inhibitor unit (TIU) per dry matter (DM), feed pea cultivars are classified into four groups: very low activity (2-4 TIU mg⁻¹ DM), low activity (4-7 TIU mg⁻¹ DM), medium activity (7-10 TIU mg⁻¹ DM) and fairly high activity (10-13 TIU mg⁻¹ DM). Due to achievements in breeding, responding to the demands by animal husbandry, all modern feed pea cultivars have low or very low trypsin inhibitor activity, making farmers independent from processing industry and providing them with an excellent source of quality plant protein. Although it is confirmed that environment may influence trypsin inhibitor activity, it is certain that genotype remains the most important factor in its expression and thus underlines the role of breeding.

Among other anti-nutritional factors in pea, there are tannins and lectins. Tannins are present in colored-flowered cultivars, that may be used for both forage and grain production, although often with decreased digestibility of grain crude protein in comparison with typical feed pea cultivars. The content of lectins is generally considered as low and thus of less importance.

Anti-Nutritional Factors in Faba Bean and Other Legumes

The main anti-nutritional factors in faba bean are tannins. Like in pea, there is a strong positive correlation between white color of flowers and reduction of tannin content, being controlled by at least two recessive genes. The cultivars without tannins, commonly known as *zero-tannin* or *tannin-free* faba bean, have found a wide application for both human consumption and in animal feeding and essentially ensured the place of faba bean in feed production. The combination of the absence of tannins and winter hardiness in faba bean proved as possible and have resulted in the development of winter tannin-free cultivars.

Among the poisonous matters that make common vetch unsuitable for monogastric animals and humans, the most important are γ -glutamyl- β -cyanoalanine, that has a bad influence on metabolism of sulphuric amino acids, and vicine and convicine, responsible for a disease called favism. Among the most important achievements of the contemporary common vetch breeding are potential for a hay crude protein content up to 260 g kg⁻¹, a dry grain crude protein content of about 300 g kg⁻¹ and less than 0.60 % of grain toxins.

The main anti-nutritional factors in lupins are alkaloids, together with phytates, protease inhibitors and lectins. The development of so-called *sweet* types of white and other lupins, meaning the selection for low-alkaloid content, have been one of the major achievements in lupin domestication. Modern sweet lupin cultivars usually have an alkaloid content of less than 200 mg kg⁻¹, with a strict regulation in great lupin producers such as Australia and Poland. However, the cultivars with high alkaloid content, called *bitter lupins*, have their role as forage or green manure crops. A neurotoxin, β -oxalylamino alanine (BOAA) play the main anti-nutritional role in grass pea and causes a disease called Lathyrism.

The Bio-Chemical and Toxicological Effects of Plant's Secondary Metabolites

Anti-nutritional factors diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities. Cyanogenic glucoside on hydrolysis yields toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems, depress growth through interference with certain essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death.

Alkaloids cause gastrointestinal and neurological disorders. The glycoalkaloids, solanine and chaconine present in potato and *Solanum spp.* are haemolytically active and toxic to fungi and humans. Some of the toxicological manifestations of potato glycoalkaloids involve gastrointestinal upsets and neurological disorders, especially in doses in excess of 20 mg/100 g sample. Coumarins, which are constituents of forage, have been associated with the so-called bleeding disease in cattle consuming spoiled or putrid sweet clover. It is believed that cinnamic acid or its derivatives are the precursors of coumarin and that when plant tissues containing derivatives are disrupted by mastication, freezing, drying or microbial spoilage, coumarin is transformed to the haemorrhagic factor, dicoumarol. Dicoumarol depresses blood prothrombin concentration and by implication reduces clotting time.

Tannins cause decreased feed consumption in animals, bind dietary protein and digestive enzymes to form complexes that are not readily digestible. They also cause decreased palatability and reduced growth rate.

Saponins cause hypercholesterolemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic to rats. Saponins from *Bulbostemma paniculatum* and *Pentapamax leschenaultii* have also been

demonstrated to have anti-spermal effects on human spermatozo.

They significantly inhibited acrosome activity of human sperms and the spermicidal effect was attributed to strong damage of the spermal plasma membrane.

Trypsin (protease inhibitor) causes pancreatic enlargement and growth depression. Haemagglutinins are proteins known for agglutinating red blood cells. They depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract.

Phytates bind minerals like calcium, iron, magnesium and zinc and make them unavailable. Oxalates, like phytates, bind minerals like calcium and magnesium and interfere with their metabolism. They also cause muscular weakness and paralysis. Oxalates also cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia. Oxalates cause nephrotic lesions in the kidney. Oxalate, phytates and tannins are anti-nutrients, which could be toxic when consumed in an unprocessed food. The bioavailability of the essential nutrients in plant foods could be reduced by the presence in these plants of some anti-nutritional factors such as oxalates and cyanogenic glycosides. Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions as the oxalate binds the calcium ions to form insoluble calcium oxalate complexes. As a result of this, people with the tendency to form kidney stones are advised to avoid oxalate-rich foods.

Gossypols are reported to cause animal and human toxicity and high incidence of irreversible testicular damage. Dietary gossypol can also bring about increased requirement, not only for lysine, but also for iron which it can chelate. At the physiological level, gossypol reduces oxygen availability in the blood, while the proteins are reduced by approximately 20% in pigs fed 0.06% free gossypol in the diet. Other physiological abnormalities include hypertrophy and dilution of heart muscles and changes in electrocardiogram. Dietary free gossypol of up to 0.02- 0.03% has been reported to cause death in growing pigs while poultry can tolerate fairly high dietary levels. Gossypol is rapidly deposited in the eggs and has been implicated in egg yolk discoloration.

Plant oestrogens also cause toxicity in animals. For example, it has long been recognized that sheep grazing subterranean clover (*Trifolium subterraneum*) are prone to poor reproductive performance. The causal chemical agent has since been identified as the isoflavone and genistein. Chronic exposure to these oestrogenic principles in plants may lead to biochemically and physiologically active levels. There is ample evidence that uncontrolled use of plant oestrogens could produce various types of tumors in animals.

There are some anti-nutritional factors in some plants, especially leguminous plants, whose mode of action is poorly understood. These are anti-vitamin factors. Raw kidney beans are believed to contain an antagonist to vitamin E as evidenced by liver necrosis in rats and muscular dystrophy and low concentration of plasma tocopherols in chicks. Anti-vitamin E has also been noted in isolated soya protein, which is suspected to be tocopherol oxidase.

Effects of different Processing Methods on the Anti-Nutritional Factors

Glucosinolate content was reduced to 40, 70 and 89% at irradiation dose levels of 10, 20 and 30 kGy, respectively ($p < 0.01$). Gamma irradiation treatment with its radiolytic effects can destroy glucosinolate molecules. The trypsin inhibitor values were significantly reduced ($P < 0.05$) by the different treatment methods, with cooking being the most effective. Soaking of the beans overnight reduced the trypsin inhibitor activities (TIA) by 6.3% and cooking of the soaked beans caused further reduction in the TIA content by 66.7%. Roasting and autoclaving significantly reduced the amount of TIA by 23.05 and 12.09%. Soaking and fermentation decreased the tannins content. These processes produce enzymes that break down complexes to release free tannins. The free tannins are leached out.

Fermentation reduced cyanide in soaked seeds. Cooking and fermentation reduced cyanide in soaked seeds to safe levels. The

HCN is soluble in soaking water as such was leached out in the atmosphere. They further reported that cooking and fermentation synergistically reduced tannins. Cooking and fermentation broke down tannin enzyme and protein-tannin complexes and released free tannins which subsequently leached out the products. The soaking, sprouting, cooking and fermentation appeared to have beneficial effects as methods of processing. Combinations of cooking and fermentation improved the nutrient quality and reduced the anti-nutritional factors inherent in sprouted cereal products to safe levels much greater than any of the other processing methods tested.

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