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Full Length Research Paper

Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects

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Anti-nutritional factors are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods and they play a vital role in determining the use of plants for humans. This paper is aimed to review the updated scientific information regarding the potential health benefits and adverse effects associated with major antinutritional factors (Tannins, Phytates, Oxalates, Saponins, Lectins, Alkaloids, Protein inhibitors, Amylase inhibitors, Cynogenic Glycosides, Chlorogenic Acids, Toxic Amino Acids, Anti-Vitamins and Goitrogens) found in plant foods. Antinutrients in plant foods are responsible for deleterious effects related to the absorption of nutrients and micronutrients. However, some antinutrients may exert beneficial health effects at low concentrations. For example, phytic acid, lectins, tannins, saponins, amylase inhibitors and protease inhibitors have been shown to reduce the availability of nutrients and cause growth inhibition. However, when used at low levels, phytate, lectins, tannins, amylase inhibitors and saponins have also been shown to reduce the blood glucose and insulin responses to starchy foods and/or the plasma cholesterol and triglycerides. In addition, phytates, tannins, saponins, protease inhibitors, goitrogens and oxalates have been related to reduced cancer risks. This implies that anti-nutrients might not always harmful even though lack of nutritive value. Despite of this, the balance between beneficial and hazardous effects of plant bioactives and anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components. Due to this, they can be considered as anti-nutritional factors with negative effects or non-nutritive compounds with positive effects on health.

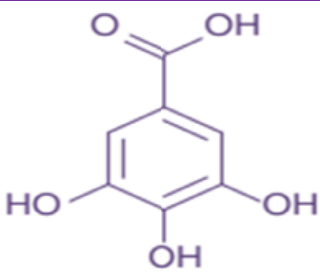
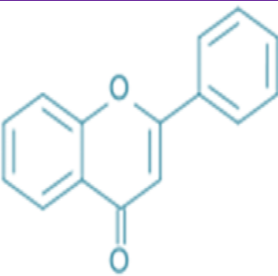
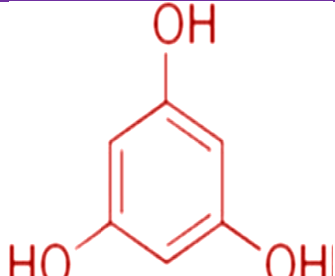
Keywords: Anti-nutritional factors, Potential Health Benefits, Adverse Health Effects, Human Foods, Plants

INTRODUCTION

Anti-nutritional factors are a chemical compounds synthesized in natural food and / or feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of food/feed) which exerts effect contrary to optimum nutrition (Soetan and Oyewole, 2009) □ Such chemical

compounds, are frequently, but not exclusively associated with foods and feeding stuffs of plant origin. These anti-nutritional factors are also known as 'secondary metabolites' in plants and they have been shown to be highly biologically active. This secondary metabolites are secondary compound produced as side products of processes leading to the synthesis of primary

Table 1. Tannins can be classified into three broad groups: hydrolysable tannins, condensed tannins and Pseudotannins.

Classification of Tannins			
Class/Polymer	Hydrolysable Tannins	Condensed Tannins	Pseudotannins
Basic Unit	 <p>Gallic Acid</p>	 <p>Flavone</p>	 <p>Phloroglucinol</p>
Source	Plants	Plants	Brown Algae

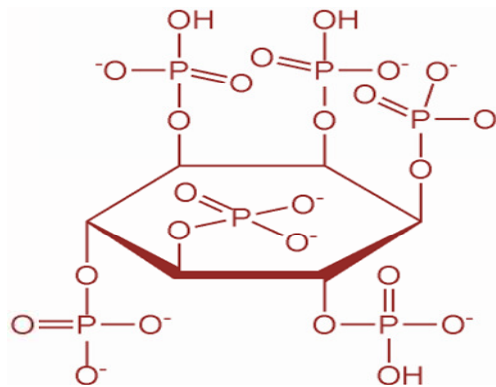


Figure 1. Structure of Phytate (Insp_6), empirical formula= $\text{C}_6\text{P}_6\text{O}_{24}\text{H}_{18}$

are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms and protect the tree and also commonly found tissues part of plants like in bud, leaf, root, seed and stem tissues. Apart from tanning, tannins are also used in dyeing, photography, refining beer and wine as well as an astringent in medicines. Significantly, tannins form a vital element of tea (Stéphane, 2004).

While soluble, astringent materials are found in some plants like tea and coffee, tannins are supplemented to various processed foods, including ice-cream and caramel. They are also used as refining materials to precipitate proteins in wines and beer. Tannins have traditionally been considered as antinutritional factors but it is now known that their beneficial or depend upon their chemical structure and dosage and the total acceptable tannin daily intake for a man is 560 mg. As tannins often lower the absorption of some materials into the body, tannins are also often known as anti-nutrients (Anonymous, 1973). The antinutritional properties of tannins depend upon their chemical structure and dosage and the total acceptable tannin daily intake for a man is

560 mg. For example, tannins are found in tea and coffee and consuming too much of these beverages without milk may lead to calcium and iron deficiency in the body and often lead to osteoporosis and anemia (Stéphane, 2004).

In order to counter these problems, it is advised that one should take tea or coffee between meals and not consecutively. In addition, adding milk or lemon juice to the tea helps in reducing or neutralizing tannins' adverse actions on iron intake. Similarly, consuming food that is rich in vitamin C also helps in neutralizing tannin's effects on iron absorption. (Osada K, and Ogino Y., 2004).

Recent studies have demonstrated that products containing chestnut tannins included at low dosages (0.15-0.2 %) in the diet can be beneficial (Schiaivone *et al.*, 2008). The most abundant polyphenols are the condensed tannins, found in virtually all families of plants, and comprising up to 50% of the dry weight of leaves. Condensed tannins inhibit herbivore digestion by binding to consumed plant proteins and making them more difficult for animals to digest, and by interfering with protein absorption and digestive enzymes. Tannins had been reported to affect protein digestibility, adversely

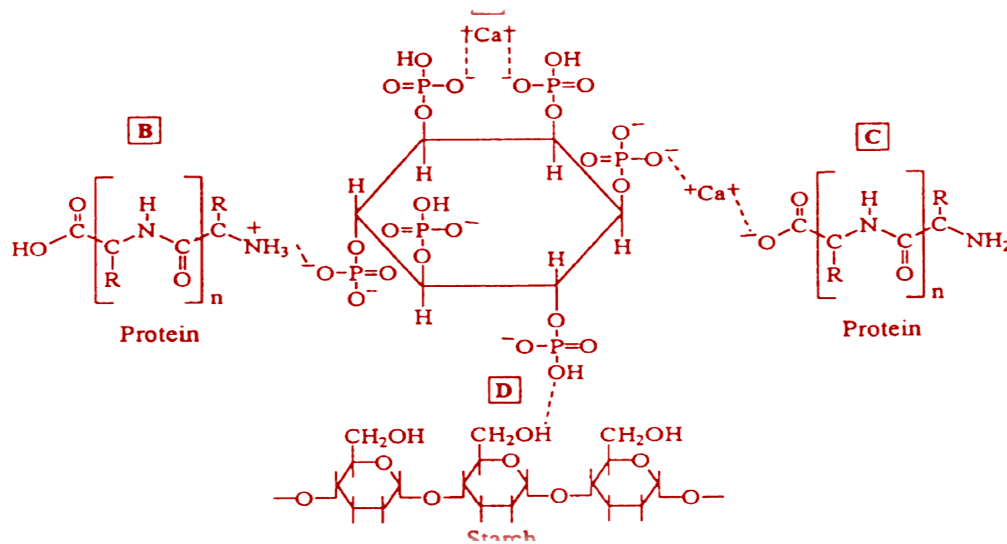


Figure 2. Possible interaction of Phytate with Minerals, Protein and Starch (Fereidoon, 2012).

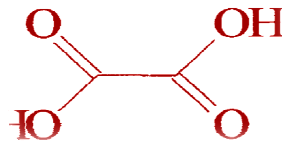


Figure 3. Structure of oxalic acid

influencing the bioavailability of non-haem iron leading to poor iron and calcium absorption, also carbohydrate is affected leading to reduced energy value of a diet containing tannins (Adeparusi, 2001).

Bressani and Elias (1980) found tannins to be heat stable and that they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. Tannins are known to be present in food products and to inhibit the activities of trypsin, chymotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (De Lumen and Salamat, 1980).

Tannins are known to be responsible for decreased feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. Makkar *et al.*, (1988) reported that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed. Tannins also form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the

antinutritional effects of tannin containing foods (Mole and Waterman, 1987).

The new technologies used to analyze molecular and chemical structures have shown that a division into condensed, hydrolyzable and Pseudo tannins is far too simplistic and readily form indigestible complexes with proteins and other macro-molecules under specific environmental conditions (Mole and Waterman, 1987).

Hydrolyzable Tannins

At the center of a hydrolyzable tannin molecule, there is a carbohydrate (usually D-glucose). The hydroxyl groups of the carbohydrate are partially or totally esterified with phenolic groups such as gallic acid (in gallotannins) or ellagic acid (in ellagitannins). Hydrolyzable tannins are hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids. Examples of gallotannins are the gallic acid esters of glucose in tannic acid (C₇₆H₅₂O₄₆), found in the leaves

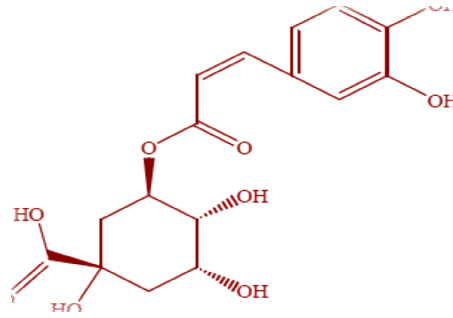


Figure 4. Structure of chlorogenic acid

and bark of many plant species.

Condensed Tannins

Condensed tannins, also known as proanthocyanidins, are polymers of 2 to 50 (or more) flavonoid units that are joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis. While hydrolyzable tannins and most condensed tannins are water soluble but few very large condensed types of tannin are insoluble (Wheeler SR., 1999).

Pseudotannins

This is not such a different group of tannins, but may be treated as sub group because they do not obey to Goldbeaters skin test and is low molecular weight compounds. Chlorogenic acid in coffee and nux vomica, ipecacuanha acid in ipecacuanha and catechins in cocoa are examples of pseudotannins. The detection test for chlorogenic acid is carried out by extracting the drug with water and treating this extract with ammonia solution, followed by exposure to air, which leads slowly to formation of green color.

Widely distributed polyphenols in plants are not directly involved in any metabolic process and are therefore considered secondary metabolites. Some polyphenolic compounds have a role as defense chemicals, protecting the plant from predatory attacks of herbivores, pathogenic fungi and parasitic weeds. Polyphenols in the grains also prevent grain losses from premature germination and damage due to moulds (FAO, 1995).

Certain polyphenoles are able to bind Fe, which make the complex-bound Fe unavailable for absorption. The amount of Fe-binding phenol galloyl groups in foods roughly corresponds to the degree of inhibition of Fe absorption. All major types of food polyphenoles can strongly inhibit dietary non-haem iron absorption. The negative influence on Fe absorption is nutritionally the most important, especially in industrial products such as

infant formulas, but more importantly in many developing countries where the diet is based on cereal and legume products. Cereals contain varying amounts of polyphenols and generally the amounts are considered higher in the colored seeds (Sandberg, 2002).

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Phytate (is also known as Inositol hexakisphosphate (InsP6)) is the salt form of phytic acid, are found in plants, animals and soil. It is primarily present as a salt of the mono- and divalent cations K^+ , Mg^{2+} , and Ca^{2+} and accumulates in the seeds during the ripening period. Phytate is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains. In addition, phytate has been suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, as a potent natural anti-oxidant (Loewus, 2002).

Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 percent (w/w) (Loewus, 2002). The phosphorus bound to phytate is not typically bio-available to any animal that is non-ruminant. Ruminant animals, such as cows and sheep, chew, swallow, and then regurgitate their food. This regurgitated food is known as *cud* and is chewed a second time. Due to an enzyme located in their first stomach chamber, the rumen, these animals are able to separate, and process the phosphorus in phytates. Humans and other non-ruminant animals are unable to do so (Weaver and Kannan, 2002).

Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn^{2+} , $Fe^{2+/3+}$, Ca^{2+} , Mg^{2+} , Mn^{2+} , and Cu^{2+} (Weaver and Kannan, 2002). Whether or not high levels of consumption of phytate-containing foods will result in mineral deficiency will depend on what else is being consumed. In areas of the world where cereal proteins are a major and predominant dietary factor, the associated phytate intake is a cause for concern (IUFoST, 2008).

Phytate markedly decrease Ca bioavailability, and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Ca: Phy is

reported to be 6:1 (Oladimeji et al., 2000). In human studies, Phy:Zn molar ratios of 15:1 have been associated with reduced zinc bioavailability, and the molar ratio $[Ca][Phy]/[Zn]$ is a better predictor of zinc availability, because calcium exacerbates phytate's effect on zinc absorption, and if the values were greater than 0.5 mol/kg, there would be interference with the availability of zinc (Oladimeji et al., 2000).

Besides, phytate has also been reported to form complexes with proteins at both low, and high pH values. These complex formations alter the protein structure, enzymatic activity, and proteolytic digestibility. The phytate degrading enzyme, phytase, is in vogue for degrading phytate during food processing, and in the gastrointestinal tract. The major concern about the presence of phytate in the diet is its negative effect on mineral uptake (Greiner and Konietzny, 2006). Also, phytate was reported to interact with carbohydrate (starch) will reduce their bioavailability and digestion. For instance, the formation of phytate-carbohydrate complex influences digestion rate of starch (Dost and Tokul, 2006).

At the same time, phytate may have beneficial roles as an antioxidant, and anticarcinogen (Jenab and Thompson, 2002). The presence of phytate in dietary sources is believed to reduce iron-induced oxidative injury and reverse stimulation colorectal tumorigenesis (tumour formation) due to its mineral chelating potential. The outcome of surveillance of populations consuming vegetarian-type diets has shown lower incidence of Cancer, which suggests that phytate has an Anticarcinogen effect that acts as anti-cancer agent against colon, soft tissues, metastatic lung cancer and mammary cancer (Shamsuddin, 2002).

Dietary phytate also may have health benefits for Diabetes patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying. Likewise, phytate has also been shown to regulate Insulin secretion (Shamsuddin, 2002). It is believed that phytate reduces Blood clots, Cholesterol, and Triglycerides, and thus prevents Heart diseases. It is also suggested that it prevents renal stone development. It is used as a complexing agent for removal of traces of heavy metal ions (Selvam, 2002). Dost and Tokul (2005) reported that phytic acid can prevent kidney stone formation. Consumption of diets rich in calcium (Ca) induces kidney calcification and subsequently renal stone development. Thus, consumption of food enriched with phytic acid able to maintain adequate Ca urinary levels to inhibit calcium oxalate crystallization. Besides, the addition of phytate into fruits, vegetables, cheese, noodles, soy sauces, juices, bread, alcoholic beverages, meats, fishmeal pastes and canned seafood increase nutritive value, prolong shelf life and prevent discolouration of food (Dost and Tokul, 2006).

Depending on the amount of plant-derived foods in the diet, and the grade of food processing, the daily intake of phytate can be as high as 4500 mg. On average, daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries, and 150–1400 mg for mixed diets (Golden, 2009). Among the cooking treatments boiling appeared effective to reduce the phytate level, which could reduce as high as 20% of phytate (Bhandari and Kawabata, 2004).

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A salt formed from oxalic acid (chemical formula $HOOC-COOH$) is known as an Oxalate: for example, Calcium oxalate, which has been found to be widely distributed in plants (Liebman, 2002). Strong bonds are formed between oxalic acid, and various other minerals, such as Calcium, Magnesium, Sodium, and Potassium. This chemical combination results in the formation of oxalate salts. Some oxalate salts, such as sodium and potassium, are soluble, whereas calcium oxalate salts are basically insoluble. The insoluble calcium oxalate has the tendency to precipitate (or solidify) in the Kidneys or in the Urinary tract, thus forming sharp-edged calcium oxalate crystals when the levels are high enough. These crystals play a role to the formation of kidney stones formation in the urinary tract when the acid is excreted in the urine (Noonan and Savage, 1999).

Higher content of oxalate can bind to calcium present in food, thereby rendering calcium unavailable for normal physiological, and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission, and as clotting factor in the blood. The calcium oxalate, which is insoluble, may also precipitate around soft tissues such as the kidney, causing kidney stones. Though loss of calcium leads to degeneration of bones, teeth, and impairment of blood clotting process (Umaru *et al.*, 2007).

When oxalic acid is consumed, it irritates the lining of the gut, and can prove fatal in large doses. Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50–60 mg per day (Massey, *et al.*, 2001). Oxalic acid is a common and wide-spread component of most plant families. While the levels of this acid in these plants are generally low, it is the high concentrations in the leaves, and cones of plants consumed daily that are of concern.

Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract (Liebman *et al.*, 2011). When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. In ruminants oxalic acid is of only minor significance as an

anti-nutritive factor since ruminal micro-flora can readily metabolize soluble oxalates, and to a lesser extent even insoluble Ca oxalate. While the importance of the anti-nutritive activity of oxalic acid has been recognized for over fifty years it may be a subject of interest to nutritionists in the future (Reyers and Naude, 2012).

The values of oxalate changes as a result of processing. Soaking and cooking of foodstuffs high in oxalate will reduce the oxalate content by leaching. Boiling may cause considerable skin rupture, and facilitate the leakage of soluble oxalate into cooking water; this may be the possible reason to observed high reduction in oxalate level upon boiling (Bhandari, and Kawabata, 2004). It is reported that boiling affects the highest reduction in oxalate (82.1% reduction after boiling for 40min as opposed to roasting and steeping those reduce 61.9% after 40-45 min and 43.3% after 24 years, respectively (Abratt and Reid, 2010).

Oxalic acid forms water soluble salts with Na^+ , K^+ , and NH_4^+ ions, it also binds with Ca^{2+} , Fe^{2+} , and Mg^{2+} rendering these minerals unavailable to animals. However Zn^{2+} appears to be relatively unaffected. In plants with a cell sap of approximately pH 2, such as some species of *Oxalis* and *rumex* oxalate exists as the acid oxalate (HC_2O_4), primarily as acid potassium oxalate. In plants with a cell sap of approximately pH 6, such as some plants of goosefoot family it exists as oxalate (C_2O_4)²⁻ ion usually as soluble sodium oxalate and insoluble calcium and magnesium oxalates. Calcium oxalate is insoluble at a neutral or alkaline pH, but freely dissolves in acid. (Noonan and Savage, 1999).

Oxalate can be found as soluble and insoluble forms in plants. Soluble salts are formed when oxalate binds with potassium, sodium and magnesium (magnesium oxalate is less soluble than the potassium and sodium salts) while insoluble salts are produced when the oxalate binds with calcium and iron. Oxalate can also be found as free oxalic acid. Cooking can reduce the soluble oxalate content of many common vegetables, but not the insoluble fraction, if the cooking water containing some of the leached soluble oxalate is discarded (Poeydomenge *et al.*, 2007).

Oxalate can be found in relatively small amounts in many plants and its distribution within plants is also uneven, in general oxalate content is highest in leaves followed by the seeds it is the lowest in the stems. Oxalic acid concentrations tend to be higher in plants than in meats, which can be considered oxalate free planning low oxalate diets. Although many plants have calcium oxalate crystals in their leaves, stems and roots, aroids are known to have these in their corms, rhizomes, etc. in extremely high concentrations (Horrocks, *et al.*, 2008).

In view of this, the importance of the oxalate content of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio, oxalate:Ca is greater than 1, since under these

circumstances the oxalate has the potential to complex not only the Ca contained in the plant but also that derived from other food sources (Noonan and Savage, 1999).

In addition to ingestion of food, mainly oxalic acid- or oxalate containing plants, these compounds may also be formed in the body as a result of the normal metabolism and biotransformation of ethylene glycol and several other compounds, such as glycolaldehyde, ascorbic acid, xylitol, glycerol, or glyoxylic acid (Horrocks, *et al.*, 2008).

In humans, the majority of oxalate is absorbed in the proximal portion of the gastrointestinal tract, with both active and passive uptake mechanisms. In food, oxalic acid is typically found as soluble sodium or potassium oxalate; insoluble calcium oxalate; or magnesium oxalate, which is also poorly soluble in water. The absorption rates of oxalate from different foods (bioavailability) range from 2 to 15%; and this process is likely dependent on a number of factors, including, among others, presence of divalent cations such as calcium and magnesium that can bind oxalate within the gastrointestinal tract, the proportion of total dietary oxalate in a soluble form under different pH conditions in the intestinal tract, and presence of oxalate-degrading bacteria in the gut (Massey, *et al.*, 2001).

Humans lack the enzymes needed to metabolize endogenous and dietary oxalate. It is well established that little oxalate catabolism occurs after absorption and >90% of absorbed oxalate can be recovered in the urine within 24-36 h after ingestion. Participation of anaerobic bacteria such as *Oxalobacter formigenes* is relevant to understand the bioavailability of oxalate for most animal species, human beings included. These intestinal bacteria can degrade up to 40% of the ingested oxalate to nontoxic substances (Liebman *et al.*, 2011).

Elimination of oxalic acid and oxalates can differ, depending on the chemical form and the metabolic processes. Therefore, insoluble calcium oxalate formed in the gastrointestinal tract can be eliminated in feces, or degraded by gastrointestinal bacteria (e.g., *O. formigenes*); and circulating oxalate can be eliminated through urine (Umaru *et al.*, 2007).

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Saponins are secondary compounds that are generally known as non-volatile, surface active compounds which are widely distributed in nature, occurring primarily in the plant kingdom. The name 'saponin' is derived from the Latin word *sapo* which means 'soap', because saponin molecules form soap-like foams when shaken with water. They are structurally diverse molecules that are chemically referred to as triterpene and steroid glycosides. They consist of nonpolar aglycones coupled with one or more monosaccharide moieties (Oleszek, 2002). This combination of polar and non-polar structural elements in their molecules explains their soap-like behaviour in aqueous solutions.

The structural complexity of saponins results in a

number of physical, chemical, and biological properties, which include sweetness and bitterness (Kitagawa, 2002), foaming and emulsifying properties, pharmacological and medicinal properties, haemolytic properties, as well as antimicrobial, insecticidal, and molluscicidal activities (Sparg et al., 2004). Saponins have found wide applications in beverages and confectionery, as well as in cosmetics (Uematsu et al., 2000) and pharmaceutical products (Sparg et al., 2004). Due to the presence of a lipid-soluble aglycone and water soluble sugar chain(s) in their structure (amphiphilic nature), saponins are surface active compounds with detergent, wetting, emulsifying, and foaming properties (Wang et al., 2005).

Saponins were treated as toxic because they seemed to be extremely toxic to fish and cold-blooded animals and many of them possessed strong hemolytic activity (Price *et al.*, 2001). Saponins, in high concentrations, impart a bitter taste and astringency in dietary plants. The bitter taste of saponin is the major factor that limits its use. In the past, saponins were recognized as antinutrient constituents, due to their adverse effects such as for growth impairment and reduce their food intake due to the bitterness and throat-irritating activity of saponins (John *et al.*, 2004). In addition, saponins were found to reduce the bioavailability of nutrients and decrease enzyme activity and it affects protein digestibility by inhibit various digestive enzymes such as trypsin and chymotrypsin (Simee W., 2011).

Saponins are attracting considerable interest as a result of their beneficial effects in humans. Recent evidence suggests that saponins possess hypocholesterolemic, immunostimulatory, and anticarcinogenic properties (Oleszek, 2002). In addition, they reduce the risk of heart diseases in humans consuming a diet rich in food legumes containing saponins. Saponin-rich foods are important in human diets to control plasma cholesterol, preventing peptic ulcer, osteoporosis and to reduce the risk of heart disease (Kao *et al.*, 2008). Saponins are used as adjuvants in viral (*e.g.*, *Quillaja saponaria*-21) and bacterial vaccine (*e.g.*, *Quillaja* saponins) applications (John *et al.*, 2004). A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning (Uematsu et al., 2000). In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones.

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Lectin comes from the Latin word “legere”, which means “to select”. Lectins have the ability to bind carbohydrates. Nowadays, proteins that can agglutinate red blood cells with known sugar specificity are referred to as “lectins” (Fereidoon S., 2014). The name “hemagglutinins” is used when the sugar specificity is unknown. Lectins and hemagglutinins are

proteins/glycoproteins, which have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharides or oligosaccharides. They can bind to the carbohydrate moieties on the surface of erythrocytes and agglutinate the erythrocytes, without altering the properties of the carbohydrates (Sze Kwan Lam & Tzi Bun Ng, 2013).

Lectins are glycoproteins widely distributed in legumes and some certain oil seeds (including soybean) which possess an affinity for specific sugar molecules and are characterized by their ability to combine with carbohydrate membrane receptors. Lectins have the capability to directly bind to the intestinal mucosa, interacting with the enterocytes and interfering with the absorption and transportation of 0.01% free gossypol within some low-gossypol cotton nutrients (particularly carbohydrates) during digestion and causing epithelial lesions within the intestine. Although lectins are usually reported as being labile, their stability varies between plant species, many lectins being resistant to inactivation by dry heat and requiring the presence of moisture for more complete destruction (Boehm and Huck, 2009).

Lectins have become the focus of intense interest for biologists and in particular for the research and applications in agriculture and medicine. These proteins with unique characteristics have found use in diverse fields of biology and as more lectins are being isolated and their role in nature elucidated, they continue to occupy an important place in agricultural and therapeutic areas of research (Sze Kwan Lam & Tzi Bun Ng, 2013).

Lectins are carbohydrate binding proteins present in most plants, especially seeds like cereals, beans, etc., in tubers like potatoes and also in animals. Lectins selectively bind carbohydrates and importantly, the carbohydrate moieties of the glycoproteins that decorate the surface of most animal cells. Dietary lectins act as protein antigens which bind to surface glycoproteins (or glycolipids) on erythrocytes or lymphocytes (Sauvion *et al.*, 2004). They function as both allergens and hemagglutinins and are present in small amounts in 30% of foods, more so in a whole-grain diet. Lectins have potent *in vivo* effects. When consumed in excess by sensitive individuals, they can cause 3 primary physiological reactions: they can cause severe intestinal damage disrupting digestion and causing nutrient deficiencies; they can provoke IgG and IgM antibodies causing food allergies and other immune responses (Boehm and Huck, 2009) and they can bind to erythrocytes, simultaneously with immune factors, causing hemagglutination and anemia. Of the 119 known dietary lectins, about half are panhemagglutinins, clumping all blood types. The remainder are blood-type specific. In general, lectins alter host resistance to infection, cause failure to thrive and can even lead to death in experimental animals (Vasconcelos and Oliveira, 2004).

Lectins are found in a wide range of vegetables and

some fruits. In humans they have been reported to cause damage, including mass food poisoning from raw or under-cooked kidney beans and hemolytic anemia and jaundice from Mexican fava beans in Glucose-6-Phosphate dehydrogenase deficient individuals (Sauvion *et al.*, 2004). Plant lectins that are not efficiently degraded by digestive enzymes and that have an affinity for the surface of gut epithelial cells, such as those present in the Leguminosae family, can be poisonous (Fereidoon S., 2014). Acute symptoms following ingestion include nausea, vomiting and diarrhea. Long-term intake in rodent models is characterized by increased cell turnover, gut hyperplasia and weight loss. Areas of epithelial cell necrosis and even zones of complete epithelial cell denudation are seen in biopsies of the stomach and intestine of mammals and insects (Sauvion *et al.*, 2004) fed plant lectins. Indeed, the plant lectin may function as a natural insecticide. Epithelial cell microvilli particularly are affected by lectin exposure, which initiates disruption and shedding of these membrane rich surface projections. Confusingly, however, when cells are treated with lectins *in vitro*, even at very high doses, necrosis is not observed, though many other responses have been noted including mitogenesis, vacuole formation and inhibition of exocytosis (Boehm and Huck, 2009).

Until recently the main use of lectins was as histology and blood transfusion reagents, but in the past few decades it has been realised that many lectins are toxic, inflammatory, or both; resistant to cooking and digestive enzymes and present in much of our food (Van Damme *et al.*, 2010). It is thus no surprise that they sometimes cause "food poisoning." But the really disturbing finding came with the discovery in 1999 that some food lectins get past the gut wall and deposit themselves in distant organs. So they could be one of the important environmental factors that cause real life diseases. The edible portions of fresh and processed foods show lectin activity including such common foods as salad ingredients, fruits, spices, dry cereals and roasted nuts. Many of the other food plants have been shown to contain phytohemagglutinins (Nachbar *et al.*, 2000). While, in most cases, the significance of the latter is somewhat obscured since the non edible parts of the plant have been tested, nevertheless, it is quite apparent that exposure to dietary lectins is a frequent and widespread event. Although both cooking and the normal digestive processes might be expected to blunt or abrogate dietary lectin activity, this need not necessarily be the case. Liener (Liener, 2003) has pointed out that dry heat may not completely destroy lectin activity. This phenomenon is clearly illustrated in the finding of hemagglutinating activity in the processed wheat germ, peanuts and dry cereals.

In addition, several of the lectins have been found to be resistant to proteolytic digestion e.g., wheat germ agglutinin, tomato lectin,

navy bean lectin and when looked for, have been recovered intact in stool (Brady *et al.*, 2007). Ingestion of lectins also has major cholecystokinin-mediated effects on gastrointestinal function and growth. It can be said that at least some lectins in foodstuffs will survive one or both degradative processes to interact with cells, secretions and microflora of the digestive tract resulting in, as yet unknown, functional consequences. Given the significant exposure of the populace to dietary lectins and the unusual breadth of biological activities potentially affected, it is obvious that future investigations of their nutritional effects will have to encompass a wider spectrum of functional parameters than heretofore tested (Boehm and Huck, 2009).

In general, the toxicity of lectins arises from their binding with the specific receptor sites on the epithelial cells of the intestinal mucosa with subsequent lesion and abnormal development of microvillae (Brady *et al.*, 2007). The consumption of lectin-containing foods may lead to endogenous loss of nitrogen and protein utilization. The carbohydrates and proteins that are undigested and unabsorbed in the small intestines reach the colon where they are fermented by the bacterial flora to short-chain fatty acids and gases. These may in turn contribute to some of the gastrointestinal symptoms associated with the intake of raw beans or purified lectins. The lectin-induced disruption of the intestinal mucosa may allow entrance of the bacteria and their endotoxins to the blood stream and cause toxic response. Lectins may also be internalized directly and cause systemic effects such as increased protein catabolism and breakdown of stored fat and glycogen, and disturbance in mineral metabolism (Fereidoon S., 2014).

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Alkaloids are one of the largest groups of chemical compounds synthesised by plants and generally found as salts of plant acids such as oxalic, malic, tartaric or citric acid. Alkaloids are small organic molecules, common to about 15 to 20 per cent of all vascular plants, usually comprising several carbon rings with side chains, one or more of the carbon atoms being replaced by a nitrogen. They are synthesized by plants from amino acids. Decarboxylation of amino acids produces amines which react with amine oxides to form aldehydes. The characteristic heterocyclic ring in alkaloids is formed from Mannich-type condensation from aldehyde and amine groups (Watkins *et al.*, 2004).

The chemical type of their nitrogen ring offers the means by which alkaloids are subclassified: for example, glycoalkaloids (the aglycone portion) glycosylated with a carbohydrate moiety. They are formed as metabolic by-products. Insects and herbivores are usually repulsed by the potential toxicity and bitter taste of alkaloids (Finotti *et al.*, 2006).

Tubers of the common potato (*Solanum tuberosum*) have a natural content of the two toxic and bitter

glycoalkaloids (GA) α-solanine and α-chaconine (Finotti *et al.*, 2006). The levels are normally low and without adverse effects on food safety and culinary quality. However, consumption of potato tubers with unusually high GA contents (300-800 mg kg⁻¹) has occasionally been associated with acute poisoning, including gastrointestinal and neurological disturbances, in man. Tuber GA levels are inheritable and can vary considerably between different species. Environmental factors experienced by tubers during germination, growth, harvest and storage may affect GA levels further (Jadhav *et al.*, 2009).

Alkaloids are considered to be anti-nutrients because of their action on the nervous system, disrupting or inappropriately augmenting electrochemical transmission. For instance, consumption of high tropane alkaloids will cause rapid heartbeat, paralysis and in fatal case, lead to death. Uptake of high dose of tryptamine alkaloids will lead to staggering gait and death. Indeed, the physiological effects of alkaloids on humans are very evident. Cholinesterase is greatly inhibited by glycoalkaloids, which also cause symptoms of neurological disorder. Other toxic action includes disruption of the cell membrane in the gastrointestinal tract (Friedman *et al.*, 2003).

Alkaline pH conditions generally enhance absorption of glycoalkaloids, where binding with sterols in cell membranes causes extra disruption. Lethal doses for humans range between 3 and 6 mg/kg body weight, although susceptibility varies considerably among individuals. A dose of more than 2 mg/kg is usually considered toxic. Korpan *et al.* (2004) identified poison symptoms as including vomiting, diarrhoea, abdominal pain, apathy, weakness and unconsciousness. Nicotine, caffeine, quinine and strychnine are well-known examples of alkaloids. Randolph (2008) claimed that alkaloids in food might well be at least partially responsible for the food-allergy effect of addition, where withdrawal from the food causes disagreeable symptoms. As an illustration, lower dose of alkaloids mediate important pharmacological activities, such as analgesic, reducing blood pressure, killing tumour cells, stimulating circulation and respiration (Simee W., 2011).

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Protease inhibitors are widely distributed within the plant kingdom, including the seeds of most cultivated legumes and cereals. Protease inhibitors are the most commonly encountered class of antinutritional factors of plant origin. Protease inhibitors have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals (Liener and Kakade, 1980). Due to their particular protein nature, protease inhibitors may be easily denatured by heat processing although some residual activity may still remain in the commercially produced products. The antinutrient activity of protease inhibitors is associated with growth inhibition and pancreatic hypertrophy. Potential beneficial effects of

protease inhibitors remain unclear, although lower incidences of pancreatic cancer has been observed in populations where the intake of soybean and its products is high (□□□□□□□□□□□□□□□□). While protease inhibitors have been linked with pancreatic cancer in animal studies, they may also act as anticarcinogenic agents. The Bowman-Birk inhibitors derived from soybean have been shown to inhibit or prevent the development of chemically-induced cancer of the liver, lung, colon, oral and esophagus (Witschi, 1999).

Trypsin inhibitor and chymotrypsin inhibitor are protease inhibitors occurring in raw legume seeds. Trypsin inhibitors that inhibit the activity of the enzymes trypsin and chymotrypsin in the gut, thus preventing protein digestion, are found in many plant species mainly in different grain legumes. Trypsin inhibitors are a unique class of proteins found in raw soybeans that inhibit protease enzymes in the digestive tract by forming indigestible complexes with dietary protein. These complexes are indigestible even in the presence of high amounts of digestive enzymes. Protease inhibitors reduce trypsin activity and to a lesser extent chymotrypsin; therefore impairing protein digestion by monogastric animals and some young ruminant animals (Liener, 2005).

Since the pancreas is responsible for the production of most digestive enzymes any substance that affects the pancreatic function will evidently influence nutrient digestibility and availability (Mushtaq, 2000). There are many plant derived trypsin inhibitors. Most of these inhibitors differ in their specificity. Many can inhibit one or two enzymes. Different forms of inhibitor may be present in the same seed, and most of the inhibitors can inhibit trypsin, but they may also inhibit chymotrypsin. The two main classes of protease inhibitors found in soybeans are Kunitz and Bowman-Birk. Bhattacharyya *et al.*, (2007) identified Bowman Birk Type inhibitors as " ... usually 8-kDa proteins with seven disulfide bridges ... " and Kunitz Type as " ... 20-kDa proteins with just two disulfide linkages".

The Kunitz trypsin inhibitors bind the trypsin enzyme in a 1:1 molar ratio. The Kunitz inhibitor family was the first family to be isolated. It is a peptide comprising 181 amino acids containing two disulfide bridges with a molecular weight of about 20-kDa. As this inhibitor inhibits trypsin stoichiometrically to form a stable complex, it is known as a single-headed inhibitor. It primarily inhibits trypsin, but it can weakly inhibit chymotrypsin. It is inactivated by heat and by gastric juices (Birk *et al.*, 2008).

In contrast, the Bowman-Birk trypsin inhibitors have two binding sites; one binds trypsin and the other binds chymotrypsin. The Bowman-Birk inhibitor family is widely distributed in legume seed. It is a smaller peptide molecule and contains 71 amino acids. It contains a high level of cystine and has seven disulfide bridges. The molecular weight is about 8-KDa. It is a double-headed

molecule and inhibits both trypsin and chymotrypsin at two different binding sites. Bowman–Birk inhibitors are resistant to gastric juices and to proteolytic enzymes. There is also a suggestion that they may be resistant to breakdown by heat (Birk et al., 2008).

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Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes. Pigeon pea have been reported to contain amylase inhibitors. These inhibitors have been found to be active over a pH range of 4.5-9.5 and are heat labile (Marshall and Lauda, 2007).

Amylase inhibitors inhibit bovine pancreatic amylase but fail to inhibit bacterial, fungal and endogenous amylase. Pigeon pea amylase inhibitors are synthesized during late seed development and also degraded during late germination. Amylase inhibitors are also very heat labile and have been reported as having hypoglycemic effects. However, instability of this inhibitor under the conditions of the gastrointestinal tract resulted in failure to reduce insulin responses and increase the caloric output of food by using them as starch blocker tablets (Giri and Kachole, 2004).

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The cyanogenic glycosides belong to the products of secondary metabolism, to the natural products of plants. These compounds are composed of an α -hydroxynitrile type aglycone and of a sugar moiety (mostly D-glucose). Cyanogenic glycosides (α -hydroxynitrile glycosides) are derived from the five protein amino acids Val, Ile, Leu, Phe and Tyr and from the nonproteinogenic amino acid cyclopentenyl glycine. Although derived from six different building blocks, they constitute a very small class with around 50 different known structures. A number of plant species produce hydrogen cyanide (HCN) from cyanogenic glycosides when they are consumed. These cyanogens are glycosides of a sugar, often glucose, which is combined with a cyanide containing aglycone. Cyanogenic glycosides are classified as phytoanticipins. Their general function in plants is dependent on activation by β -glucosidases to release toxic volatile HCN as well as a ketones or aldehydes to fend off herbivore and pathogen attack (Zagrobelyny, et al., 2004).

Cyanogenic glycosides or cyanoglycosides account for approximately 90% of the wider group of plant toxins known as cyanogens. The key characteristic of these toxins is cyanogenesis, the formation of free hydrogen cyanide, and is associated with cyanohydrins that have been stabilised by glycosylation (attachment of sugars) to form the cyanogenic glycosides. Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the $\text{Fe}^{3+}/\text{Fe}^{2+}$ contained in the enzyme. This causes a decrease in the utilization of oxygen in the tissues. Cyanide causes an

increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism. Cyanide activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle. Hydrogen cyanide will reduce the energy availability in all cells, but its effect will be most immediate on the respiratory system and heart (Jones et al., 2000).

Cyanogenic glycosides are widely distributed in the plant kingdom and more than 2500 different plant species have been reported to contain cyanogenic glycosides including cassava (*Manihot esculenta*), linseed (*Linum usitatissimum*), various sorghums (*Sorghum* spp) and white clover (*Trifolium repens*). Lesser quantities are found in the kernels of such plants as almonds (*Amygdalus communis*), apricots (*Prunus armeniaca*), peaches (*Prunus persica*), and apples (*Malus sylvestris*) (Zagrobelyny, 2008).

When plant material containing the glycoside is consumed, it is broken down by a β -glucosidase to produce a sugar and an aglycone. The aglycone is then acted upon by a hydroxynitrile lyase to produce cyanide and an aldehyde or a ketone. As cyanide is extremely toxic, one of the most obvious symptoms is death. In the body, cyanide acts by inhibiting cytochrome oxidase, the final step in electron transport, and thus blocks ATP synthesis. Prior to death, symptoms include faster and deeper respiration, a faster irregular and weaker pulse, salivation and frothing at the mouth, muscular spasms, dilation of the pupils, and bright red mucous membranes (Bjarnholt, 2008).

In Africa, where many people consume cassava on a regular basis, many members of the human population are regularly exposed to low levels of cyanide in their diet. This is associated with a condition called tropical ataxic neuropathy. Symptoms include neurological disturbances, which affect vision, hearing, and the peripheral nervous system. There are also raised levels of blood thiocyanate and goiter. Cassava consumption, combined with protein deficiency, which is often common in societies that consume large amounts of starchy tubers, can lead to reduced glucose tolerance and diabetes. There is some evidence that the symptoms can be partially alleviated by the administration of vitamin B12 and methionine (Tattersall et al., 2001).

The toxicity of a cyanogenic plant depends primarily on the potential concentration of hydrogen cyanide that may be released upon consumption. If the cyanogenic plant is inadequately detoxified during processing or preparation of the food, the potential hydrogen cyanide concentration which may be released can still be high. Upon consumption of a cyanogenic plant, β -glucosidase will be released during digestion and remain active until deactivated by the low pH of the stomach. This enzyme will hydrolyse the cyanogenic glycoside and release at

and quantitative must be used in making judgments about the food value of particular plant species. The fact that a plant or part of a plant is eaten by human is only an indication of acceptability. Antinutritional factors in foods are responsible for the deleterious effects that are related to the absorption of nutrients and micronutrients which may interfere with the function of certain organs. Most of these antinutritional factors are present in foods of plant origin. Thus, the presence of cyanogenic glycosides, enzyme inhibitors, lectins, Tannins, alkaloids, and Saponins in foods may induce undesirable effects in humans if their consumption exceeds an upper limit. Certain harmful effects might also be due to the breakdown products of these compounds. However, some antinutritional factors as well as their breakdown products may possess beneficial health effects if present in small amounts. The mechanism through which the antinutritional and beneficial effects of food antinutritional factors are exerted is the same. Thus, manipulating processing conditions, in addition to removing certain unwanted compounds in foods, may be required to eliminate the deleterious effects of antinutritional factors and take advantage of their health benefits. Health risk factors associated with antinutritional factors include: lack of knowledge of the tolerance levels to these compounds in the human organism, little available information on the degree of variation of individual risks and little knowledge with respect to the influence of environmental factors on the detoxification capacity of the human organism.

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