

Pulse Phytonutrients: Nutritional and Medicinal Importance

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Abstract: Pulses are important food crops which offer significant nutritional and health advantages due to their high protein content and a unique nutritional profile, i.e., low fat source of digestible protein, dietary fibre, complex carbohydrates, resistant starch and a number of essential vitamins, especially, the B-group vitamin B9 (folate). In addition to these vitamins and minerals contributing to a healthy diet, pulses contain a number of non-nutritive bioactive substances including enzyme inhibitors, lectins, saponins, phytates, phenolic compounds and oligosaccharides. The latter contributes beyond basic nutritional value and is particularly helpful in the fight against non-communicable diseases often associated with diet transitions and rising incomes. Phytic acid exhibits antioxidant activity and protects DNA damage, phenolic compounds have antioxidant and other important physiological and biological properties, and galacto-oligosaccharides may elicit prebiotic activity. Research findings on different phytochemicals in pulse seeds and their role in preventing the lifestyle diseases has been discussed. Encouraging awareness of the nutritional value of pulses can help consumers adopt healthier diets and also could be an important dietary factor in improving longevity.

Keywords: Pulses, polyphenols, carotenoids, saponins, tocopherols, folates.

INTRODUCTION

Pulses are annual leguminous crops that yield grains or seeds of variable size, shape and colour within a pod, used for both food and feed. Dietary patterns are changing all over the world and the share of non-cereal foods in the total calorie and protein consumption is increasing. Diets based on pulse crops as nutrient-rich whole food are gaining attention with respect to combating non-communicable diet-related diseases, including obesity, diabetes, cardiovascular diseases, and different types of cancer. Non-communicable diseases are a global health concern that affects more than one in every ten adults [1]. The relationship between diet and health is an active area of research; a substantial body of evidence already indicates that food components can influence physiological processes [2]. Thus, functional foods are of increasing interest in the prevention and/or treatment of diseases [3]. Pulses are rich source of protein, dietary fibre, complex carbohydrates, resistant starch and a bevy of vitamins and minerals such as folate, selenium, potassium, Fe and Zinc. The food values of pulse seeds are high providing 336 to 354 Kcal of energy per 100 g, which is about the same calorific value per unit weight as cereals. Their protein content is generally about 2-3 times that of most cereals (Table 1). The Food and Agriculture Organization (FAO) estimates that 850 million people worldwide suffer from under nutrition, to which insufficient protein in the diet is a significant contributing factor. Pulse seeds contain 17-35 % of protein on a dry weight basis. In terms of

solubility in specific solvents, pulse proteins fall primarily into the albumin (water-soluble) and globulin (salt-soluble) classes. The storage proteins legumin and vicillin are globulins, and the albumins comprise the heterogeneous group of enzymes, amylase inhibitors, and lectins. In general, macronutrient studies have shown that protein is more satiating than carbohydrates or lipids [4]. Moreover, the protein in pulses has been implicated in providing satiety [5]. Thus, pulse proteins as consumed may contain bioactive components that contribute to satiety and weight management.

Most grains have a poor balance of essential amino acids. The cereals (maize, wheat, rice, etc.) tend to be low in lysine (Lys), whereas legumes are often low in the sulfur-rich amino acids methionine (Met) and Cystine (Cys) [6]. Consumption of cereal-based foods with pulse ingredients has the potential to increase the protein quality of the overall food product which potentially can help to prevent malnutrition in developing countries, especially among children. Diets comprising of High-Lys and high-Met cereals and legumes could allow diet formulations that reduce animal nitrogen excretion by providing an improved balance of essential amino acids. The mono and oligosaccharides represent only a small per cent of total carbohydrate in pulses, whereas, starch is the most abundant carbohydrate. Starches account for 22–45% of pulse grain weight depending on the source. As is typical of other grains, pulse starches are composed of amylose, a linear α -1,4-linked glucan with few branches in the molecular weight range of 10^5 – 10^6 , and amylopectin, a highly branched and much larger molecule (molecular weight 10^7 – 10^9) composed of α -

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Table 1: Energy and Protein Content of Major Pulse and Cereal Crops

Crop category	Scientific name	Common name	(Value /100g)	
			Kcal	Protein
Pulses	<i>Vigna radiata</i>	Mung bean	347	23.86
	<i>Vigna mungo</i>	Black gram	341	25.21
	<i>Vigna unguiculata</i>	Cowpeas	336	23.52
	<i>Vicia faba</i>	Faba bean	341	26.12
	<i>Cicer arietinum</i>	Chickpea	364	19.30
	<i>Lens culinaris</i>	Lentil	353	25.80
	<i>Cajanus cajan</i>	Pigeon pea	343	21.70
Cereals	<i>Triticum durum</i>	Wheat, durum	339	13.68
	<i>Triticum aestivum</i>	Wheat, bread	340	10.69
	<i>Zea mays</i>	Maize	365	9.42
	<i>Oryza sativa</i>	Rice, medium grain	360	6.61
	<i>Pennisetum glaucum</i>	Millet	378	11.02
	<i>Sorghum</i>	Sorghum	339	11.30
	<i>Hordeum vulgare</i>	Barley	352	9.91

Source: FAOSTAT.

1,4-linked glycosyl units of varying lengths connected by α -1,6 branch points. Pulse starches generally have a higher content of amylose compared with cereal and tuber starches; this factor plus their associated high capacity for retrogradation may reduce the starch digestion rate, rendering them either slowly digestible and/or resistant to digestion. All pulses have a low glycemic index (GI) (i.e. the carbohydrate is slowly digested). For example, compared to white bread with a GI value of 100, the approximate GI values for chickpea are 40, lentil 42 and pea 45, while GI for beans can vary from 40 to 55. They are low in fat and contain no cholesterol. Pulses contain antioxidants (e.g. vitamin E), selenium, phenolic acids, and phytic acids. Pulses also contain other non-nutritive compounds such as enzyme inhibitors, lectins, prebiotic carbohydrate, galacto-oligosaccharides and resistant starch, polyphenols, phytates and saponins, that were earlier considered as anti-nutrient factors (ANF's) affecting the digestibility and bioavailability of nutrients in humans and animals.

This review highlights the current status of knowledge regarding the bioactive components present in the seeds of different pulse crops and focuses predominately on the recent advances in their health prevention activities.

NUTRITIVE BIOACTIVE COMPONENTS IN PULSES: ROLE IN HUMAN NUTRITION AND HEALTH

Slow Digestible Starch and Resistant Starch

Pulses have a unique profile of low digestible carbohydrates including several healthful prebiotic

compounds: raffinose-family oligosaccharides (RFO), fructooligosaccharides (FOS), sugar alcohols, and resistant starch. α -Galactosides are neither absorbed nor hydrolyzed in the upper gastro-intestinal tract of humans, accumulating in the large intestine of the human digestive system. Human beings lack α -galactosidase, the enzyme that is responsible for degrading these oligosaccharides [7]. Therefore, α -galactosides undergo microbial fermentation by colonic bacteria resulting in the production of hydrogen, methane and carbon dioxide, major components of flatulent gases leading to abdominal discomfort [8]. The α -galactosides, are derived from sucrose and have 1–3 α -1,6-linked galactosyl units attached. They are commonly known as raffinose (1 galactosyl unit), stachyose (2 galactosyl units), and verbascose (3 galactosyl units). In chickpeas the raffinose content ranges from 0.48-0.73 g /100 g dry weight and stachyose content ranges from 1.76-2.72 g /100 g dry weight, however verbascose was not detected [9]. Ciceritol and stachyose, two important galactosides in chickpeas, constitute 36-43% and 25%, respectively, of total sugars (mono-, di-, and oligosaccharides) in chickpeas seed [10]. Fructans are an important ingredient in functional foods because evidence suggests that they promote a healthy colon (as a prebiotic) and help reduce the incidence of colon cancer. Fructans consisting of linear β -(1 \rightarrow 2)-linked fructose polymers are called inulins. Fermentation of prebiotics and certain low-digestible carbohydrates elicits a variety of health effects which can be subdivided into two main groups: functional effects and disease risk reduction. The functional effects are

physiological effects that can be measured relatively easily, including induction of satiety [11], reduction of caloric intake [12], reduction of serum cholesterol, triglycerides, and lowering of glucose concentration [13]. Disease risk reduction, as implied by the name, is the effect over time of one or more functional effects to reduce the risk/severity of chronic diseases. For example, prebiotic-induced satiety leading to reduced caloric intake and improved serum lipid profile all contributes to reducing the risk and /or the severity of obesity and metabolic syndrome [14].

Dietary Fiber

Fiber is a group of substances that is chemically similar to carbohydrates. Non-ruminant animals including humans poorly metabolize fiber for energy or other nutritional uses. Dietary fiber has been recognized as a healthy food component [15]. It consists of a mixture of polymeric non-starch substances, which resist enzymatic digestion in the human gastrointestinal tract. Most of these substances are complex carbohydrates like cellulose, hemicellulose and pectin. There are many health benefits which are associated with adequate intake of these substances including, lowering of blood cholesterol and sugar levels, reduced risk of constipation, obesity, diabetes, cardiovascular complications, colon and rectal cancer, gallstones, piles and hernia. The dietary fibers can be classified into soluble and insoluble. Soluble fiber is digested slowly in the colon whereas the insoluble fibre is metabolically inert and aid in bowel movement. The lignin, cellulose and some hemicellulose typically constitute the insoluble dietary fiber (IDF), whereas pectin, hemicellulose and other non-starch dietary fiber polysaccharides make up the soluble dietary fiber (SDF). Legumes seeds typically contain more dietary fiber than cereals and are better sources of metabolically active SDF. In their raw state, pulses are high in fiber, with 15–32% total dietary fiber; of this, approximately one-third to three-quarters is insoluble fiber and the remaining is soluble fiber. Insoluble fiber is associated with fecal bulking through

its water-holding capacity, whereas soluble fiber ferments, positively affecting colon health through production of SCFA, lowered pH, and potential microbiota changes. Viscous soluble fiber (pectin and few other non-starch polysaccharides) may increase gastric distension and help to slow gastric emptying rate. The soluble fiber being viscous, liner along the walls of the intestine, reduces glucose and cholesterol absorption into the blood stream [16]. Since legumes are better source of soluble fiber than cereals, they are particularly recommended in the diets of both diabetic and patients with cardiovascular problems [17]. Public health organizations recommended that adults should take 25 to 35 g dietary fiber per day. The dietary fiber content of grain legumes are presented in Table 1. It is evident that the total dietary fiber (TDF) content of legume seeds varied from 11.5 to 33.2% (Table 2). Total dietary fibre content (DFC) in chickpea ranges from 18-22 g /100 of raw chickpea seed [18]. Soluble and insoluble DFC is about 4-8 and 10-18 g 100⁻⁹ of raw chickpea seed, respectively [19].

Vitamin B9 (Folic Acid)

Folic acid, also known as vitamin B₉, or folacin and folate, as well as pteroyl-L-glutamic acid, pteroyl-L-glutamate, and pteroyl-monoglutamic acid is required to synthesize DNA, repair DNA, and methylate DNA. Tetrahydrofolate and derivatives, collectively called folates, are water-soluble B vitamins. It also helps in maturation of red blood cells and prevents anaemia. Folate deficiency is a global problem affecting millions of people in both developed and developing countries. Inadequate intake of folic acid during pregnancy increases the risks of preterm delivery, low birth weight, fetal growth retardation, and developmental neural tube defects (NTDs). In addition, low folate intake and elevated homocysteine levels are associated with the occurrence of neurodegenerative disorders, cardiovascular diseases, and a range of cancers. On the other hand, adequate intake of both folates and folic acid in diets decreases total homocysteine levels in plasma. Humans and animals cannot synthesize

Table 2: Dietary Fibre Content in Major Pulses

Sr. No.	Crop	Insoluble Dietary Fibre (% dry wt.)	Soluble Dietary fibre (% dry wt.)	Total Dietary Fibre (% dry wt.)
1.	Pea (green)	10.2	3.2	13.4
2.	Lentil	9.5	2.0	11.5
3.	Chickpea	17.2	5.5	22.7
4.	Pigeonpea	12.2	3.5	15.5

Source: Khan *et al.*, (2007). Dietary Fibre Profile of Food legumes. S. J. Agric. Vol. 23, No. 3, 2007.

folates, and therefore they must be supplied from plant-based and animal foods including liver and eggs. Most staple food crops, including cereals, potato (*Solanum tuberosum* L.), and banana (*Musa* sp), are poor sources of dietary folates. Diets which are based on these foods often do not reach the folate RDA of 400 µg/day. Generally, leafy vegetables contain more folates than roots and fruits. The USDA nutrient database shows that lentils (*Lens culinaris* L.) and common beans (*Phaseolus vulgaris* L.) are two pulses that are rich in folates. Although pulses are very good source of folates, but are not readily available due to complex binding with bio-molecules [20]. There are also reports that chickpeas have higher content of folate compared with pea [21]. Folate content in raw chickpeas and peas were 149.7 and 101.5 µg/100 g, respectively, and 78.8 and 45.7 µg/100 g in boiled chickpeas and peas, respectively, indicating that some folate may have leached in the water used for processing.

Non- Nutritive Bioactive Components in Pulses: Role in Human Nutrition and Health

In addition to the above nutritive compounds, pulse seeds contain a number of compounds which qualify as phytochemicals with significant potential benefits to human health (for example, as anticarcinogenic, hypocholesterolemic or hypoglycemic agents). Antinutritional compounds vary considerably in their biochemistry. They can be proteins (protease inhibitors, α-amylases, lectins), glycosides (α-galactosides, vicine and convicine), tannins, saponins, alkaloids. Hence, methods for their extraction, determination and quantification are specific for each compound. They do not appear equally distributed in all pulses, and their physiological effects are diverse. Some of these compounds are important in plant defence mechanisms against predators or environmental conditions. Others are reserve compounds, accumulated in seeds as energy stores in readiness for germination. Processing generally improves the nutrient profile of legume seed by increasing *in vitro* digestibility of proteins and carbohydrates and at the same time there are reductions in some antinutritional compounds. Most antinutritional factors are heat-labile, such as protease inhibitors and lectins, so thermal treatment would remove any potential negative effects from consumption. On the other hand tannins, saponins and phytic acid are heat stable but can be reduced by dehulling, soaking, germination and/or fermentation. However, in order to exert an effect, either local or systemic, these substances have to survive at least to some extent the digestive process within the

gastrointestinal tract. The scientific interest in non-nutritional factors is now also turning to studies of their possible useful and beneficial applications as gut, metabolic and hormonal regulators and as probiotic/prebiotic agents. In this new era of intense bioactive research, the same ANF's are undergoing a reappraisal. Many of these non-nutritive bioactive components have been found to have positive health effects associated with their consumption. Phytic acid exhibits antioxidant activity and protects DNA damage, phenolic compounds have antioxidant and other important physiological and biological properties, saponins have hypocholesterolaemic effect and anti-cancer activity. Pulses are gluten-free - they offer a great variety for those on a gluten-free diet (e.g. for Celiac disease, a gastro-intestinal disorder). The on-going research is examining how whole pulses and the individual components offer protective and therapeutic effects in such chronic health conditions such as obesity, cardiovascular disease, diabetes and cancer and how consumption of legumes could potentially let people live longer.

Polyphenolic Compounds

The major polyphenolic compounds of pulses consist mainly of tannins, phenolic acids and flavonoids. The seed's color of pulses is mainly due to the presence of polyphenolic compounds viz., flavonoids such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins). The pulses with the highest polyphenolic content are dark, highly pigmented varieties, such as red kidney beans (*Phaseolus vulgaris*) and black gram (*Vignamungo*). Condensed tannins (proanthocyanidins) have been quantified in hulls of several varieties of field beans (*Vicia faba*) and are also present in pea seeds of colored-flower cultivars. Lentils have the highest phenolic, flavonoid and condensed tannin content (6.56 mg gallic acid equivalents / g, 1.30 and 5.97 mg catechin equivalents / g, respectively), followed by red kidney and black beans [22]. The seed coat in lentil is very rich in catechins, procyanidins dimers and trimers. It was reported that the major monomeric Xavan-3-ol was (+) catechin-3-glucose, with lesser amounts of (+)-catechin and (-)-epicatechin [23]. Until recently, phenolic compounds were regarded as non-nutritive compounds and it was reported that excessive content of polyphenols, in particular tannins, may have adverse consequences because it inhibits the bioavailability of iron [24] and thiamine [25] and blocks digestive enzymes in the gastrointestinal tract [26]. Phenolic compounds can also limit the bioavailability of proteins

with which they form insoluble complexes in the gastrointestinal tract. Later on the significance of phenolic compounds was gradually recognized and several researches have now reported that phenolics offer many health benefits and are vital in human nutrition [27]. Pulses with highest total phenolic content (lentil, red kidney, and black beans) exert the highest antioxidant capacity assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), and the oxygen radical absorbance capacity (ORAC) [22]. Chickpea contains a wide range of polyphenolic compounds, including flavonols, flavone glycosides, flavonols, and oligomeric and polymeric proanthocyanidins. Total phenolic content in chickpea ranges from 0.92 to 1.68 mg gallic acid equivalents /g (22). The chemical and cellular antioxidant activities and phenolic profiles of 11 lentil cultivars was investigated and was found that five phenolic acids of the benzoic types and their derivatives (gallic, protocatechuic, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic acid, and protocatechualdehyde) and four phenolic acids of the cinnamic type (chlorogenic, *p*-coumaric, *m*-coumaric, and sinapic acid) as well as two flavan-3-ols [(+)-catechin and (-)-epicatechin] and one flavone (luteolin) were detected in all lentil cultivars [28]. Among all phenolic compounds detected, sinapic acid was the predominant phenolic acid, and (+)-catechin and (-)-epicatechin were the predominant flavonoids. The chemical profile of lentil (*Lens culinaris* Medik.) cultivars was analysed [29]. Extracts (100% methanol and methanol-water (1:1)) were analyzed by RP-HPLC. Chromatographic separations of the methanol extract afforded several compounds including the novel 4-chloro-1-*H*-indole-3-*N*-methylacetamide as well as itaconic acid, arbutin, gentisic acid 5-*O*-[β -D-apiofuranosyl-(1 \rightarrow 2)- β -D-xylopyranoside], and (6*S*,7*Z*,9*R*)-9-hydroxymegastigma-4,7-dien-3-one-9-*O*- β -D-apiofuranosyl-(1 \rightarrow 2)- β -D-gluco-pyranoside.

Flavonoids

Flavonoids comprise the most common group of plant polyphenols. More than 5,000 different flavonoids have been described in literature. The six major subclasses of flavonoids include the flavones (apigenin, luteolin), flavonols (quercetin, myricetin), flavanones (naringenin, hesperidin), catechins or flavanols (epicatechin, gallic acid), anthocyanidins (cyanidin, pelargonidin), and isoflavones (genistein, daidzein). Interest in the possible health benefits of flavonoids has increased owing to their potent antioxidant and free-radical scavenging activities

observed *in vitro*. Isoflavones are diphenolic secondary metabolites and have been isolated from a wide variety of plants, though the isoflavones are largely reported from the Fabaceae/Leguminosae family. According to the USDA survey on isoflavone content, lentils do not contain significant amounts of these isoflavones [30]. Two important phenolic compounds found in the chickpea are the isoflavones, biochanin A [5, 7-dihydroxy-4'-methoxyisoflavone] and formononetin [7-hydroxy-4'-methoxyisoflavone] [31]. The other phenolics detected in chickpea are daidzein, genistein, matairesinol, and secoisolariciresinol [32]. The concentration of biochanin A is higher in kabuli seeds [1420-3080 μ g/100g] compared to the desi type seeds [838 μ g/100g] [33]. There are many biological activities associated with the isoflavones, including a reduction in osteoporosis, cardiovascular disease, prevention of cancer and for the treatment of menopause symptoms [34].

Antioxidant Activity in Pulses

In the past few years, the antioxidant properties of food have been extensively studied. Excessive production of free radicals / reactive oxygen species (ROS) and lipid peroxidation are widely believed to be involved in the pathogenesis of diseases such as cardiovascular diseases, cancers, autoimmune disorders, rheumatoid arthritis, various respiratory diseases, cataract, Parkinson's or Alzheimer's diseases and also ageing. Beninger and Hosfield [35], showed that pure flavonoid compounds such as anthocyanins, quercetin glycosides and protoanthocyanidins (condensed tannins), present in the methanol extract of the seed's coat and tannin fractions from 10 colored genotypes of common bean *Phaseolus vulgaris*, displayed antioxidant activity, while the highest activity was obtained with extracts rich in condensed tannins. The Total antioxidant capacity (TAC) value for chickpea was reported as 10.7 \pm 1.3 mmol Trolox/kg [36]. Lentil showed a high total antioxidant capacity (TAC) value probably related to the high content of condensed tannins present in lentil [23]. Xu and Chang (28), reported that caffeic acid, catechin, epicatechin, and total flavonoids significantly ($p < 0.05$) correlated with peroxy radical scavenging assay in lentil cultivars. Sreeramulu *et al.* [37] evaluated the antioxidant activity of pulses, commonly consumed in India and assessed the relationship with their total phenolic content. The total phenolic content (TPC) in pulses ranged from 62.35 to 418.34 mg/100g. Among the pulses blackgram dhal had the highest TPC (418.34 mg/100g), while green gram dhal had the least

(62.35 mg/100g). The antioxidant activity as determined by three different methods showed a wide range of values. DPPH radical scavenging activity (1.07 TE/g), FRAP (373 $\mu\text{mol/g}$) and reducing power (4.89 mg/g), all three were highest in Rajmash. It was further showed that in pulses the total phenolic content (TPC) was poorly correlated with antioxidant activity (AOA), suggesting thereby that only TPC might not contribute significantly to the antioxidant activity in pulses [37].

Enzyme Inhibitors

Protein inhibitors of hydrolases present in pulses are active against proteases, amylases, lipases, glycosidases, and phosphatases. From the nutritional aspect, the inhibitors of the serine proteases trypsin and chymotrypsin found in plant foodstuffs are the most important [38]. Beans are the second largest group of seeds after cereals reported as natural sources of α -amylase inhibitors [39]. Protease inhibitors isolated from pulses are generally classified into two families, referred to as Kunitz and Bowman-Birk on the basis of their molecular weights and cystine contents. Kunitz type inhibitors have a molecular mass of ~ 20 kDa, with two disulfide bridges, and act specifically against trypsin. Bowman-Birk type inhibitors with a molecular mass of 8–10 kDa, have seven disulfide bridges, and inhibit trypsin and chymotrypsin simultaneously at independent binding sites. Protease inhibitors interfere with digestion by irreversibly binding with trypsin and chymotrypsin in the human digestive tract. They are resistant to the digestive enzyme pepsin and the stomach's acidic pH. In common bean, lima bean, cowpea, and lentil, protease inhibitors have been characterized as members of the Bowman-Birk family [38;39]. In pea, large genetic variability is available for the activity of Bowman-Birk trypsin/chymotrypsin inhibitor proteins. Protease inhibitor content is moderate in kidney bean and cowpea (8 and 10.6 g of trypsin and 9.2 g of chymotrypsin inhibited kg^{-1} , respectively) [40]. The content of α -amylase inhibitors differs greatly among legumes, with the highest amount found in dry bean.

Lectins

Lectins are proteins or glycoproteins that agglutinate erythrocytes of some or all blood groups *in vitro* depending on their specificity and high binding affinity for a particular carbohydrate moiety on the cell surface [41]. Lectins can reduce the digestibility and biological value of dietary proteins and inhibit the growth of experimental animals [42]. These

antinutritional effects are most likely caused by some lectins that can impair the integrity of the intestinal epithelium [43] and thus alter the absorption and utilization of nutrients [44]. The administration of lectins to experimental animals can also alter the bacterial flora [45]. Lectin is one of the major proteins found in lentil (*Lens culinaris*). Studies have suggested that lectins affect the immune response against ovalbumin and may promote the development of food allergy to plants containing lectins. Cooking effectively removes trypsin inhibitor and lectin of vegetable peas and significantly reduces protein and amino acid solubility [46]. Lectin can be completely removed from lentil flour after 72 h fermentation at 42 °C with a flour concentration of 79 g / L [47]. Amount of lectin in pulses vary significantly [48]. High level of lectins has been reported in kidney beans (840×10^{-5} hemagglutinating activity units (HU) /kg) and very low amount in cowpea (3×10^{-5} HU /kg). Dietary lectins have generally been considered to be toxic and anti-nutritional factor. However, many lectins are non-toxic, such as those from lentil, pea, chickpea and faba bean. *Vicia faba* agglutinin (VFA), a lectin present in broad bean, aggregated, stimulated the morphological differentiation, and reduced the malignant phenotype of colon cancer cells [49]. Inclusion of raw kidney bean in the diet of obese rats reduced lipid accumulation that was related to a decrease of insulin level caused by lectins. However, no body or muscle protein losses occurred, even at high doses, as with normal rats, suggesting a possible use of lectins as therapeutic agents to treat obesity [50]. Lectin from kidney bean seeds directly inhibits HIV-1 reverse transcriptase, an enzyme crucial for HIV replication, as well as β -glucosidase, which has a role in HIV-1 envelope protein *gp120* processing, therefore a very potent element of the antiretroviral chemotherapy.

Phytosterols

200 different types of phytosterols have been reported in plant species. In pulses, phytosterols are present in small quantities, and the most common phytosterols are β -sitosterol, campesterol, and stigmasterol [51] (Figure 1). These compounds are also abundant as sterol glucosides and esterified sterol glucosides, with β -sitosterol representing 83% of the glycolipids in defatted chickpea flour [10]. Total phytosterol content detected in the legumes ranged from 134 mg /100 g (kidney bean) to 242 mg /100 g in pea [52]. Total β -sitosterol content ranged from 160 mg /100 g (chickpea) to 85 mg /100 g (butter bean). Chickpea and pea contained high levels of campesterol

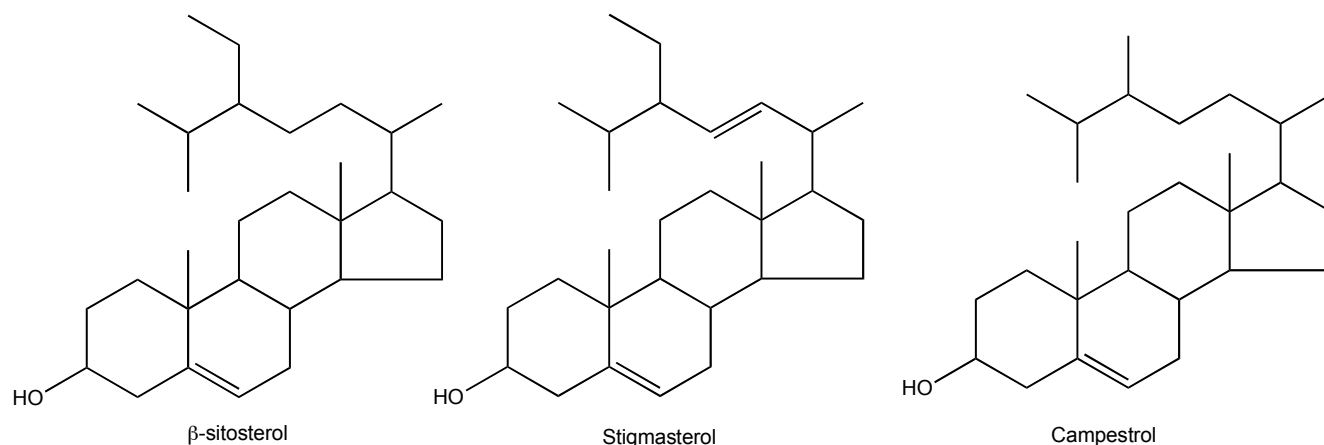


Figure 1: Major sterols reported in Pulse grains.

(21.4 and 25.0 mg /100 g, respectively). Stigmasterol content is higher in butter beans (86 mg /100 g) and squalene content in pea (1.0 mg /100 g). Weihrach and Gardner (52), reported 127 mg /100 g phytosterol level for kidney bean, with much lower concentration of phytosterols in chickpea (35 mg /100 g). The consumption of pulse grains has been reported to lower serum cholesterol and increase the saturation levels of cholesterol in the bile. A dietary study conducted on humans over a seven week period showed that serum LDL cholesterol was significantly lower during the consumption of a diet consisting of beans, lentil and field pea [53]. The study showed that consumption of pulses lowers LDL cholesterol by partially interrupting the enterohepatic circulation of the bile acids and increasing the cholesterol saturation by increasing the hepatic secretion of cholesterol. The study concluded that other pulse components in the diet may also have contributed to the observed effect; in particular, saponins, which are hydrolyzed by intestinal bacteria to diosgenin, may have exerted a positive effect [54]. Several studies have demonstrated the efficacy of plant sterols and stanols in the reduction of blood cholesterol levels, and plant sterols are increasingly being incorporated into foods for this purpose [55].

Phytic Acid

Phytic acid (myo-inositol hexaphosphate or InsP_6), a major phosphorus storage form in plants, and its salts are known as phytates, regulate various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear messenger RNA export and potentially hormone signalling important for plant and seed development [56], as well as animal and human nutrition [57]. It is often regarded as an antinutrient because of strong mineral, protein and starch binding

properties thereby decreasing their bioavailability [58]. Phytates play important role in plant metabolism, stress and pathogen resistance in addition to their beneficial effects in human diets by acting as anticarcinogens or by promoting health in other ways such as in decreasing the risk of heart disease or diabetes [59]. Wholegrain cereals and pulses have a high content of phytate [60]. In pulse seeds, phytate is located in the protein bodies in the endosperm. Phytate occurs as a minerals complex, which is insoluble at the physiological pH of the intestine (61). Raw lentil contained 0.3 mmol/kg of InsP_3 . The most abundant inositol phosphate in raw, dry legume is InsP_6 , accounting for an average of 83% of the total inositol phosphates, ranging from 77% in chickpea to 88% in black bean. The InsP_6 concentration tends to be higher in raw dry bean, blackeye peas, and pigeon peas than in lentils, green and yellow split peas, and chickpeas and ranged between 14.2 and 6 mmol /kg in black beans and chickpeas, respectively [62]. Varietal and agronomic factors, alone and in combination, often result in a wide variation in phytate content of mature legume seeds and cereal grains [63] (Table 3).

In vivo and *in vitro* studies have demonstrated that inositol hexaphosphate (InsP_6 , phytic acid) exhibits significant anticancer (preventive as well as therapeutic) properties. It reduces cell proliferation and increases differentiation of malignant cells with possible reversion to the normal phenotype and is involved in host defense mechanism, and tumor abrogation [64]. InsP_6 has been suggested to be responsible for the epidemiological link between high-fiber diets (rich in InsP_6) and low incidence of some cancers. Phytic acid delays postprandial glucose absorption, reduce the bioavailability of toxic heavy metal such as cadmium and lead, and exhibit antioxidant activity by chelating iron and copper [65]. Dietary and endogenous

Table 3: Trypsin Inhibitor Activity in Grain Legume Seeds

Sr. No.	Crop	Trypsin inhibitor activity (TIU/mg)	Reference
1.	Pea	1-14.6 1.9-6.8 6-15	Bastianelli <i>et al.</i> , 1998 Gabriel <i>et al.</i> , 2008 Guillamon <i>et al.</i> , 2008
2.	Lentil	1-9-2.8 3-8	Wang <i>et al.</i> , 2009 Guillamon <i>et al.</i> , 2008
3.	Chickpea (Desi lines) Chickpea (Kabuli lines)	12.7 10.3 15-19	Singh and Jambunathan, 1981 Guillamon <i>et al.</i> , 2008

phytic acid have protective effects against cancer and heart disease and may be responsible for the cancer-protective effects of high-fibre foods [66]. The anticarcinogenic properties of phytic acid may result from numerous factors, including its ability to chelate metal ions; this depends on the phytate retaining its integrity in the colon [67]. The backbone of most inositol phosphates in cells is *myo-inositol*. Inositol phosphates from seeds are a significant food source of *myo-inositol*, as are the phospholipids and free inositol from many plant- and animal-based foods [68]. *Myo-inositol* has been evaluated for its ability to improve the mental health of patients with various psychiatric disorders [69]. In addition to *myo-inositol*, smaller amounts of *epi-* and *scyllo-inositol* are present in human brains. *Myo-inositol* and InsP₆ have synergistic or additive effects in inhibiting the development of cancer [70]. In mice, dietary *myo-inositol* has been shown to be effective in preventing cancer of the lung [71], fore stomach [72], liver [73] colon, mammary gland, prostate, and skin [74].

Saponins

Saponins are glycosidic compounds, which are structurally composed of a lipid-soluble aglycon consisting of either a sterol or more commonly a triterpenoid and water soluble sugar residues differing in type and amount of sugars. Their biological activity is closely related to chemical structures that determine the polarity, hydrophobicity and acidity of compounds. Saponins have long been considered undesirable due to toxicity and their haemolytic activity. Although these toxicological properties of plant saponins have long been recognized, there is a renewed interest in these biologically active plant components as recent evidence suggests that saponins possess hypocholesterolemic [75], anti-carcinogenic [76] and immune-stimulatory properties [77]. There is enormous structural diversity within this chemical class, and only a few are toxic [78]. Most of the saponins occur as

insoluble complexes with 3-*b*-hydroxysteroids; these complexes interact with bile acid and cholesterol, forming large mixed micelles [75]. In addition, they form insoluble saponin–mineral complexes with iron, zinc, and calcium [79], hence their lower nutrient availability [80]. The most widely studied saponins in legumes include the soyasaponins, which are classified into group A, B, and E saponins on the basis of the chemical structure of the aglycone [81]. Chickpeas contain only one major saponin, belonging to the soyasaponin group B, which is characterized by a reducing sugar 2,3-dihydro-2,5-dihydroxy-6-methyl- H-pyran-4-one (DDMP) moiety on C-22 [82]. The DDMP is a heat-sensitive residue that provides the saponin unique characteristics, including antioxidant capacity, characteristic absorption spectrum and sweet taste. Field peas were initially thought to contain soyasaponin I (S-I) and then soyasaponin VI (S-VI) as the only soyasaponin, but recently field pea extracts were shown to contain dehydrosoyasaponin I (D-I) as a minor component [83]. D-I from pea has insecticidal and anti-feedant properties against stored product insect pests. This triterpenoid Saponin dehydrosoyasaponin I is a natural product present in chickpea and other legumes and is known to be a potent calcium-activated potassium channel opener and as such can be used for treating cardiovascular, urological, respiratory, neurological, and other disorders. Soybean and chickpea constitute major sources of saponins in the human diet [84]. Saponins have been reported in many pulses, lentils [85], and chickpeas [86], as well as in various beans, and peas [78].

Saponin content in chickpea (56 g/kg) is higher than other pulses like green gram (16 g/ kg), lentil (3.7-4.6 g /kg), fababean (4.3 g/ kg) and broadbean (3.5 g/ kg) [87]. Saponin content may vary even among the same species, because of variations in cultivars, varieties, locations, irrigation condition, type of soil, climatic

Table 4: Range of Variation Reported for Phytic Acid in Major Pulses

Sr. No.	Crop	Phytic acid (g/kg)	Reference
1.	Pea	1.3-10.2 0.2-1.3%	Bastianelli <i>et al.</i> , 1998 Champ, 2002
2.	Lentil	6.2-8.8 0.15-2.34%	Wang <i>et al.</i> , 2009 Champ, 2002
3.	Chickpea (Desi lines) Chickpea (Kabuli lines) Chickpea	7.7-12.3 5.4-11.7 0.4-1.1 %	Chitra <i>et al.</i> , 1995 Champ, 2002
4.	Mungbean	10.2-14.8	Chitra <i>et al.</i> , 1995
5.	Pigeonpea	6.8-14.9 9.9-16.4	Chitra <i>et al.</i> , 1995 Singh, 1999
6.	French bean	0.2-1.9%	Champ, 2002

Table 5: Saponin Content in Major Pulses

Sr. No.	Crop	Saponin (g/kg)	Reference
1.	Pea	1.1 0.3-1.0	Koslovska <i>et al.</i> , 2001 Bastianelli <i>et al.</i> , 1998
2.	Lentil	1.1	Koslovska <i>et al.</i> , 2001
3.	Chickpea	2.3	Koslovska <i>et al.</i> , 2001

conditions, and year during which they are grown [88]. Chickpeas, black gram, moth bean, broad beans and peas can contain 3.6, 2.3, 3.4, 3.7, and 2.5 g kg⁻¹ dry matter of saponins, respectively [88]. Saponin content in dehulled light and dark coloured peas range from 1.2 to 2.3 g/ kg dry matter [89]. The Saponin content varies from 0.3 to 1.1 g/kg in peas (Table 4). Some saponin is lost during processing as has been reported in moth beans [88], black gram [90] and pigeon pea [91].

Foods rich in saponins are reported to reduce plasma cholesterol by 16-24 % [92]. The mechanism of cholesterol reduction is by binding to dietary cholesterol or bile acids, thereby increasing their excretion through faeces [93]. β -sitosterol (dominant phytosterol in chickpea) is helpful in decreasing serum cholesterol levels and incidence of coronary heart disease [94]. Recent evidence suggests that legume saponins may possess anti-cancer activity [22] and is beneficial for hyperlipidemia [78]. In addition, they reduce the risk of heart diseases in humans consuming a diet rich in food legumes containing saponins [95]. Epidemiological studies suggest that saponins may play a role in protection from cancer [78]. Metastatic events are critical in cancer proliferation, and there is evidence that glycosylation is an important event in this process. It has recently been demonstrated [96] that

soyasaponin I decreases the expression of R-2, 3-linked sialic acid on the cell surface, which in turn suppresses the metastatic potential of melanoma cells. The observed anticancer activity may therefore in part be due to this observed sialyltransferase inhibition activity. Additional mechanistic studies indicate that there is evidence for saponin regulation of the apoptosis pathway enzymes (AKT, Bcl, and ERK1/2), leading to programmed cell death of cancer cells [97]. Research on colon cancer cells suggests that it is the lipophilic saponin cores that may be responsible for the biological activity.

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