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Non-Nutritive Bioactive Compounds in Pulses and Their Impact on Human Health: An Overview

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ABSTRACT

This review describes the non-nutritive biologically active components in grain legumes and discusses about the bioactivity of phenols, isoflavones, phytosterols, phytic acid, saponins, tannins, protease inhibitors and bioactive complex carbohydrates in different pulse grains. These bioactive components have wide ranging biological activities and consequently many different targets and mechanism of action. The potential beneficial effect of these compounds especially their antioxidant properties and their role in the prevention of non-communicable chronic diseases such as coronary heart disease, stroke, cancer and diabetes has been discussed.

Keywords: Pulses; Grain Legumes; Bioactive Compounds; Anti Nutritional Compounds; Phenolics; Antioxidants; Antioxidant Activity

1. Introduction

The term pulses is limited to legume crops harvested solely for dry grains and excludes legumes used for oil extraction (soybean & groundnut) and those harvested green for food (green peas and green beans). The major pulses used for human consumption include chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), lentil (*Lens culnaris*), green gram (mung bean) (*Vigna radiata*), black gram (urdbean) (*Vigna mungo*), fieldpea (*Pisum sativum*), lupin (*Lupinus* spp.), kidney bean (*Phaseolus vulgaris*), lima bean (*Vigna lunatus*), adzuki bean (*Vigna angularis*), Rice bean (*Vigna umbellata*), moth bean (*Vigna acontifolia*), dry broad bean (*Vicia faba*) and dry cowpea (*Vigna unguiculata*). Recent researches have associated the consumption of pulses with a decreased risk for a variety of chronic degenerative diseases such as cancer, obesity, diabetes and cardiovascular diseases [1-3]. Pulse grains are rich source of protein, dietary fibre, complex carbohydrates, resistant starch and a number of vitamins and minerals *viz*., folate, potassium, selenium and zinc. In addition to the macronutrients, pulses contain a wide variety of non-nutritive bioactive components such as enzyme inhibitors, phytic acid, lectins, phytosterols, phenolic compounds and saponins [4]. These non-nutritive bio-active compounds earlier considered as anti-nutrients because of their activity to reduce protein digestibility [5] and mineral bioavailability have recently been shown to have health protective effects [6]. Phytic acid exhibits antioxidant activity and protects DNA damage [7], phenolic compounds have antioxidant and other important physiological and biological properties [8], saponins have hypocholesterolaemic effect and anticancer activity [9]. This review seeks to discuss and document the potential benefits to human health derived from the consumption of pulse grains and examine the bioactivity of pulse lectins, phytic acid, isoflavones, phytosterols and saponins, and their role in the prevention of various chronic diseases.

2. Non-Nutritive Bioactive Components in Pulses

The nutritional factors in pulse grains are widely considered critical for human nutrition, however, the overwhelming evidence from epidemiological studies indicate that diets rich in pulse grains are also associated with a lower risk of several degenerative diseases, attributed to the fact that these foods supply several non-nutritive bioactive compounds/health-promoting mixture of phytochemicals which act as natural antioxidants and and protects DNA damage (**Table 1**).

2.1. 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 are the most important [10]. Common beans (*Phaseolus*

Table 1. Major non-nutritive bioactive phytochemicals in pulses.

Sr. No.	Bioactive components	Potential biological effects/health effects
1	Protease inhibitors	Reduces digestibility and bioavailability of nutrients
2	Amylase inhibitors	Reduces utilization of dietary starch and protein, Potentially therapeutic in diabetes.
3	Lectins	Can reduce the digestibility and biological value of dietary proteins.
		Can impair the integrity of the intestinal epithelium and thus alter the absorption and utilization of nutrients
		Protective effect on oxidative DNA damage and cancer chemoprevention
4	Phytates	Decreases mineral bioavailability
		Acts as anticarcinogen.
		Antioxidant activity and protects DNA damage
5	Phenolic compounds	Antioxidant activity, Has been inversely associated with the risk of colon cancer
6	Phytosterols	Lowers serum cholesterol level
7	Saponins	A potent calcium-activated potassium channel opener..
		Hypocholesterolaemic effect, Anti-cancer activity

vulgaris) are the second largest group of seeds after cereals reported as natural sources of *α*-amylase inhibitors [11]. In common beans, lima bean, cowpea, and lentil, protease inhibitors have been characterized as members of the Bowman-Birk family [10,11]. 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), and low in lupin seeds (1.1 g of trypsin and 1.4 g of chymotrypsin inhibited kg^{-1}) [12].

2.2. Lectins

Lectins or haemagglutinins are found in most plant foods [13], however, grain legumes are the main sources of lectins in human food. Beans (most species, including *Phaseolus vulgaris*) seems to be important source of lectins. Lectins from some of the pulses can inhibit the growth of experimental animals and reduce the digestibility and biological value of dietary proteins [14]. These antinutritional effects are most likely caused by some lectins that can impair the integrity of the intestinal epithelium and thus alter the absorption and utilization of nutrients. Lectin can be completely removed from lentil flour after 72 h fermentation at 42˚C with a flour concentration of 79 $g{\cdot}L^{-1}$ [15]. High level of lectins has been reported in kidney beans (840×10^{-5} hemagglutinating activity units (HU) kg^{-1}) and very low amount in cowpea and lupin seeds (3×10^{-5} $HU{\cdot}kg^{-1}$) [13]. Studies have suggested that lectins affect the immune response against ovalbumin and may promote the development of food allergy to plants containing lectins. Lectin is one of the major proteins found in lentil (*Lens culinaris*). It accounts for about 2.4% - 5% of the total protein (17% - 23%) in kidney bean seeds, 0.8% in lima bean protein (21%), and around 0.6% of the total protein (24% - 25%) in garden pea [16].

2.3. Phytosterols

In pulses, phytosterols are present in small quantities, and the most common phytosterols are *β*-sitosterol, campesterol, and stigmasterol [17]. These compounds are also abundant as sterol glucosides and esterified sterol glucosides, with *β*-sitosterol representing 83% of the glycolipids in defatted chickpea flour [18]. Total phytosterol content detected in the legumes ranged from 134 mg/100g (kidney bean) to 242 mg/100g (pea) [19]. Total *β*-sitosterol content ranged from 160 mg/100g (chickpea) to 85 mg/100g (butter bean). Chickpea and pea contained high levels of campesterol (21.4 and 25.0 mg/100g, respectively). Weihrauch and Gardner [20], reported 127 mg/100g phytosterol level for kidney bean, with much lower concentration of phytosterols in chickpea (35 mg/100g). 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 by Duane [21], 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. The study showed that consumption of pulses lowers LDL cholesterol by partially interrupting the entrohepatic circulation of the bile acids and increasing the cholesterol saturation by increasing the hepatic secretion of cholesterol. Several studies have demonstrated the efficacy of plant sterols and stanols in the reduction of blood cholesterol levels, and plant sterols are increasingly incorporated into foods for this purpose [22].

2.4. Phytic Acid

Phytic acid (myo-inositol hexaphosphate or $InsP_6$), is a major phosphorus storage form in plants, and its salts known as phytates regulate various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear messenger RNA export and potentially hormone signaling important for plant and seed development [23]. It is often regarded as an antinutrient because of strong mineral, protein and starch binding properties thereby decreasing their bioavailability [24]. Phytate play important role in plant metabolism, stress and pathogen resistance in addition to their beneficial effects in human diets by acting as anticarcinogen [25] or by promoting health in other ways such as in decreasing the risk of heart disease or diabetes [26]. Pulses have high content of phytate, which is located in the protein bodies in the endosperm. Raw lentil contained 0.3 $\text{mmol}\cdot\text{kg}^{-1}$ of InsP_3 [27]. 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 [28]. The InsP_6 concentration tends to be higher in raw dry bean, blackeye pea, and pigeon pea than in lentil, green and yellow split pea, and chickpea and ranged between 14.2 and 6 $\text{mmol}\cdot\text{kg}^{-1}$ in black bean and chickpea, respectively [28]. *In vivo* and *in vitro* studies have demonstrated that inositol hexaphosphate (InsP_6, phytic acid) exhibits significant anticancer (preventive as well as therapeutic) properties [29]. The anticarcinogenic properties of phytic acid may result from numerous factors. The backbone of most inositol phosphates in cells is *myo-inositol. Myo-inositol* and InsP_6 have synergistic or additive effects in inhibiting the development of cancer [30]. In mice, dietary *myo*inositol has been shown to be effective in preventing cancer of the lung [30], fore stomach [30], and liver [30,31].

2.5. Phenolic Compounds

Phenolic compounds are one of the biggest groups of nonessential dietary compounds. The bioactivity of phenols is attributed to their ability to chelate metals, inhibit lipid peroxidation and scavenge free radicals [32]. According to Rice-Evans *et al.* [33], the antioxidant properties of phenolics occur mainly due to their potential for ox-reduction, which enables them to act as reducing agents, donating hydrogen and neutralizing free radicals. The major phenolic compounds of pulses consist mainly of tannins, phenolic acids and flavonoids. The seed color of pulses is mainly due to the presence of polyphenolic compounds viz., flavonoids such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) [34]. The pulses with the highest polyphenolic content are dark, highly pigmented varieties, such as red kidney beans (*Phaseolus vulgaris*) and black gram (*Vigna mungo*) [35]. Lentil has the highest phenolic, flavonoid and condensed tannin content (6.56 mg gallic acid equivalents g^{-1}, 1.30 and 5.97 mg catechin equivalents g^{-1}, respectively), followed by red kidney and black beans [35]. Lentil showed a high Total Antioxidant Capacity (TAC) probably related to the high content of condensed tannins present in lentil [36]. The seed coat in lentil is very rich in catechins, procyanidins dimers and trimers. It was reported that the major monomeric flavan-3-ol was (+) catechin-3glucose, with lesser amounts of (+)-catechin and (−)-epicatechin [37]. Until recently, phenolic compounds were regarded as non-nutritive com- pounds and it was reported that excessive content of polyphenols, in particular tannins, may have adverse consequences because it inhibits the bioavailability of iron and blocks digestive enzymes in the gastrointestinal tract [35]. 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 [36]. High correlations between phenolic compositions and antioxidant activities have been reported [37]. Pulses with highest total phenolic content (lentil, red kidney and black bean) exert the highest antioxidant capacity assessed by 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), and the oxygen radical absorbance capacity (ORAC) [38]. 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^{-1} [39]. Xu and Chang [38], investigated the chemical and cellular antioxidant activities and phenolic profiles of 11 lentil cultivars and found that five phenolic acids of the benzoic types and their derivates (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. Among all phenolic compounds detected, sinapic acid was the predominant phenolic acid, and (+)-catechin and (−)-epicatechin were the predominant flavonoids. Tsopmo and Muir [37]) studied the chemical profile of lentil (*Lens culinaris* Medik.) cultivars. 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→2)-*β*-D-xylopyranoside], and (6*S*,7*Z*,9*R*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-gluco-pyranoside.

2.6. Isoflavones

Isoflavones are largely reported from the Fabaceae/Leguminosae family. According to the USDA survey, lentil does not contain significant amounts of these isoflavones [40]. Chickpea contains daidzein, genistein, and formononetin (0.04, 0.06, and 0.14 mg/100g, respectively), and approximately 1.7 mg/100g biochanin A [40]. Soybean has significantly higher levels of daidzein and genistein (47 and 74 mg/100g, respectively) but contain less formononetin and biochanin A as compared to chickpea. There are many biological activities associated with the isoflavones, including a reduction in osteoporosis, cardiovascular disease and prevention of cancer and for the treatment of menopause symptoms [41].

2.7. Saponins

Saponins are 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. Saponins lower nutrient availability [42] and decrease enzyme activity [43] contributing to a growth-retarding effect in animals [44]. There is enormous structural diversity within this chemical class, and only a few are toxic [9]. There is renewed interest in these biologically active plant components because recent evidence suggests that saponins possess hypocholesterolemic [44], anti-carcinogenic [45] and immune-stimulatory properties [46]. Soybean and chickpea constitute major sources of saponins in the human diet [47]. Saponins have been reported in many pulses, lupin [48], lentil [49], and chickpea [50], as well various beans, and pea [9]. Chickpea, black gram, moth bean, broad beans and peas can contain 3.6, 2.3, 3.4, 3.7, and 2.5 $g \cdot kg^{-1}$ dry matter of saponins, respectively [50]. Saponin content in dehulled light and dark colored peas ranges from 1.2 to 2.3 $g \cdot kg^{-1}$ dry matter [51]. Some saponin is lost during processing as has been reported in moth bean [52], black gram [53] and pigeon pea [54].

3. Antioxidant Phytochemicals in Pulse Grains

In the past few years, the antioxidant properties of food has been extensively studied since excessive production of free radicals/reactive oxygen species (ROS) and lipid peroxidation are widely believed to be involved in the pathogenesis of many diseases such as cardiovascular diseases, cancers, autoimmune disorders, rheumatoid arthritis, various respiratory diseases, cataract, Parkinson's or Alzhemier's diseases and also ageing. Evidence further suggests that plant derived antioxidants such as flavonoids, and related phenolic compounds plays a crucial role in the prevention of these chronic diseases [36]. Beninger and Hosfield [55], showed that pure flavonoid compounds such as anthocyanins, quercetin glycosides and protoanthocyanidins (condensed tanins), present in the seed coat methanol extract and tannin fractions from 10 colored genotypes of common bean *Phaseolus vulgaris*, all 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 ± 1.3 mmol Trolox/kg [56]. Xu and Chang, [57] reported that caffeic acid, catechin, epicatechin, and total flavonoids significantly ($p < 0.05$) correlated with peroxyl radical scavenging assay in lentil cultivars. Sreeramulu *et al* [58], 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. The black gram dhal had highest TPC (418.34 mg/100g), while green gram dhal had the least (62.35 mg/100g). In the same study [58], the antioxidant activity as determined by three different methods showed a wide range of values viz., DPPH radical scavenging activity (1.07 TE/g), FRAP (373 umol/g) and reducing power (4.89 mg/g), all three were highest in Rajmash. Sreeramulu *et al.* [58] further showed that in pulses the total phenolics content (TPC) was poorly correlated with antioxidant activity (AOA), suggesting thereby that TPC might not contribute significantly to the AOA in pulses.

Effect of Processing of Pulse Grains on Antioxidant Activity

The Antioxidant activity from foods can be influenced by the methods applied for its consumption. Various steps of processing result in significant decrease in total phenolic content (TPC) and DPPH free radical scavenging activity (DPPH) [57]. The same study also revealed that soaking and atmospheric boiling treatments decreased, while pressure boiling and steaming increased the oxygen radical absorbing capacity (ORAC). Steaming treatments resulted in a greater retention of TPC, DPPH, and ORAC values as compared to boiling treatments. However, TPC and DPPH in cooked lentil differed significantly between atmospheric and pressure boiling. Pressure processes significantly increased ORAC values in both boiled and steamed pulses compared to atmospheric processes. Greater TPC, DPPH, and ORAC values were detected in boiling water than in soaking and steaming water. Steam processing exhibited several advantages in retaining the integrity of the legume appearance and texture of the cooked product, shortening process time, and greater retention of antioxidant components. Segev *et al.* [39], studied the total phenolic content and antioxidant activity of chickpea (*Cicer arietinum* L.) as affected by soaking and cooking and reported that chickpea lines with colored testa (seed coat) contain high levels of polyphenolic compounds that exhibit high levels of antioxidant activity. They further re- ported that common processing procedures, such as soak- ing and cooking, may decrease the level of these bioactive compounds and subsequent overall antioxidant activity. Here, the effects of soaking, cooking and steaming processes were examined in relation to total phenolic content (TPC), total flavonoid content (TFC) and ferric reducing ability of plasma antioxidant activity (FRAP AA) of colored chickpea seeds. All processing steps significantly reduced TPC, TFC and FRAP AA in all of the tested chickpea seeds. Nevertheless, soaking the seeds at room temperature (for 22 h) resulted in a smaller decrease in TPC, TFC and FRAP AA than soaking at 60˚C (for 2 h). Moreover, steaming was superior to cooking in terms of conserving polyphenol and antioxidant activity. Based on these results, it was suggested that soaking at room temperature for 22 h followed by steaming for 1 h is the best method for retaining TPC, TFC and FRAP AA of colored chickpea.

4. Pulses and Human Health

The consumption of pulse grains has been reported to lower serum cholesterol and increase the saturation levels of cholesterol in the bile. Total serum cholesterol was reduced by 7%, LDL cholesterol by 6% and serum triacylglycerols by more than 17%, with no significant change in HDL-cholesterol [3]. Legume consumption of four times or more per week compared with less than once a week, was associated with 22% lower risk of CHD, and 11% lower risk of CVD [59]. Further the replacement of refined rice with whole grain and legume powder as a source of carbohydrate in a meal showed significant beneficial effects on glucose, insulin, and homocysteine concentrations and lipid peroxidation in coronary artery disease (CAD) patients, these effects are likely to substantially reduce the risk factors for CAD and diabetes [60]. The effect was primarily attributed to the content of soluble dietary fibre which has been shown to reduce total and low-density lipoprotein, cholesterol levels, as well as insulin resistance [61]. Among the food legumes, chickpea is the most hypocholesteremic agent. Dietary supplementation with chickpea resulted in significant reductions in serum total and low-density lipoprotein cholesterols in adult woman and men [62]. A recently published meta-analysis concluded that a higher intake of folate (0.8 mg folic acid) would reduce the risk of ischaemic heart disease by 16% and stroke by 24% [63]. The B-vitamin, folate has been shown to reduce homocysteine concentration, where elevated homocysteine levels have been identified as significant risk factor for increased risk of cardiovascular diseases [64]. Pea, chickpea and mungbean protein hyrolysates have been shown to have angiotensin converting enzyme (ACE) inhibitory activity [65]. Since ACE plays a key role in modulating blood pressure, ACE inhibitors, including those derived from pulses, may improve cardiovascular health [65]. Pulses are low glycemic index (GI) foods with GI values ranging from 28 - 52 [66]. The low GI in pulses is due to abundance of non-starch polysaccharides, resistant starch and oligosaccharides [67]. A decrease of blood glucose response has also been attributed to phytic acid, lectins, amylase inhibitors, or polyphenol compounds in pulse seeds [68]. A substantial increase in dietary intake of pulses as replacement food for more rapidly digested carbohydrate might therefore be expected to improve glycemic control and thus reduce incident diabetes [69]. Jenkins *et al.* [70] have shown that low GI diets resulted in moderately reduced level of Hemoglobin (Hb) A_{1c} (a glycosylated protein) which is an indicator of glucose level in blood. Further a meta-analysis of 11 studies has revealed that low GI diets resulted in decrease in mean blood glucose levels, a decrease in HbA_{1c} and improved plasma lipid parameters compared with high GI diets [71]. Recently Villegas *et al.*, [72] reported that adherence to vegetables and pulses were inversely associated with the risk of type 2 diabetes in a large Chinese population. Subjects consuming fibre-rich beans, and legumes, had the lowest BMI, smallest waist circumference (WC), and the smallest mean annual increase in BMI and improved satiety [73]. The low-GI diet (high in wholegrain bread and beans and with less white bread and rice) resulted in improved glycemic control and greater weight loss [74].

Inverse correlations between pulse consumption and colon cancer mortality and risks of prostrate cancer, gastric cancer and pancreatic cancer have been reported in several epidemiological studies [75]. Adebamowo *et al.* [76] reported that bean or lentil intake is associated with a lower risk of breast cancer. Pulses are excellent source of B-vitamin folate [77], which may play a protective role against colorectal, cervical, breast and pharyngeal cancers [78]. Selenium, primarily due to its potent antioxidant effect, appears to have a protective effect against colorectal, prostrate and lung cancers [79]. Pulses are major source of saponins, which also have antioxidant effect, exhibits direct and selective cytotoxic action against cancer cells [80]. Pea protease inhibitors also show promise as cancer chemoprventive agents [81]. The non-digestible carbohydrate in pulses (insoluble dietary fiber, oligosaccharides, resistant starch) [82] stimulates growth and/or activity of bacteria such as *bifidobacteria* and *lactobacilli* in the colon, resulting in increased formation of butyrate, a short chain fatty acid with demonstrated anti-tumor and anti-inflammatory activity [83]. In addition, pulses, particularly common bean, contain a number of polyphenols, with antioxidant and anti-mutagenic activities that could inhibit the formation of tumors [55]. In a large prospective cohort study a reduced breast cancer risk was associated with higher intake of pulses [84]. Other studies revealed that consumption of pulses such as dried beans, split peas, or lentils was negatively associated with risk of colorectal adenoma [85].

5. Conclusion

Pulses make a major share of the human diet in many locations of the world and play a significant role in the human nutrition, especially as source of protein, vitamins, minerals, dietary fibre and folic acid. Besides these important nutrients, the pulse grains also contain certain biologically active components including enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds. Some of these substances have been considered as antinutritional factors due to their effect on diet quality. On the other hand, these same compounds may have protective effects. Important biological activities have now been suggested for these bioactive compounds like enhancement of the antioxidant, antimutagenic, anticarcinogenic and antihyperglycemic effects, which makes pulses an important crop for human health.

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