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Phenolics Content in Legume and Their Health Benefit: A Review

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Review Article

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Abstract: Legumes provide good source of protein, complex carbohydrate, dietary fibre, essential vitamins and minerals and are used as food and feed purposes. In most of the developing countries, animal protein is expensive and as an alternative, legumes are consumed majorly as a source of protein. They further complement proteins from other plant sources, such as, cereal grains where they contribute essential amino acids to the diets in different parts of the world. Aside the nutritional content, they also possess non-nutrients components otherwise known as the bioactive compounds, which include inhibitors of proteases and amylases, lectins, saponins, phytic acid and phenolic compounds. Out of all these bioactive compounds, phenolic compounds are of great importance because, apart from contributing to the seed colour and sensory characteristics of the seed, they also provide several biological properties with proposed health-related benefits. Phenolic compounds are natural bioactive compounds found in legumes used for combating free radicals and reducing the oxidative damage responsible by chronic diseases.

Keywords: Legume, carbohydrate, dietary fibre, essential vitamins.

INTRODUCTION

Legumes have played a major role in the traditional diets of many regions throughout the world and are important sources of macronutrients and micronutrients. They have been recognized as functional foods apart from their nutritional value where they promote both good health and have therapeutic properties [1]. The interest gained by legumes over the years, have been because of their excellent sources of bioactive compounds which can be important sources of ingredients for uses in functional foods and other applications.

The consumption of legumes with high phenolic content has been reported in many epidemiological studies to reduce incidence of diseases such as cancer, ageing, diabetes and cardiovascular disease [2]. Polyphenols, phytic acid, tannins and saponins found in legumes have been hypothesised to prevent these chronic diseases [3].

Types of Legumes

Legume is a plant in the family Fabaceae or Leguminosae. They are arable crops, grown largely for their seed called pulse and utilised as valuable ingredients of various products for human consumption. They are also used for livestock forage and silage, and as soil - enhancing green manure [4]. There are different types of legume namely:

(a) Grain legumes which include beans, lentils, pea and pea nut are used for human consumption or oil production for industrial uses [5].
(b) Forage legumes are those that can be sown in pasture and grazed by livestock e.g Alfafa [6]
(c) Fallow/ green manure legume species are those having bacteria with special ability of fixing atmospheric nitrogen into the soil [7].
(d) Flowering legumes are grown specifically for their flowers and an example of this is Lupin. They are commercially cultivated for their blooms as well as being popular in garden worldwide [8].
(e) Industrially farmed legumes such as Indigofera and Acacia species are grown for dye and natural gum production [9].
(f) Legume species grown for timber production worldwide including Acacia Specie [10].

Nutrient composition of legumes

Nutritional compositions of legume vary according to cultivar, location of growth, climate, environmental factors, and soil type in which legumes are grown [11, 12]. These nutritional compositions consist of high level of proteins, fats, carbohydrates, dietary fibers, B- group vitamins (thiamin, riboflavin, niacin) and minerals [13]. Their protein content has been reported to vary between 15 and 40 g/100 g [14] and they contribute to the essential amino acid in the diet of millions of people, especially the vegetarians [15]. Legume's proteins are usually used to complement cereals, root and tuber proteins in most African countries [16]. Its seeds are also rich in polyunsaturated fatty acids in addition to other micro and macronutrients [1, 17]. Table 1 shows the nutritional composition of cooked pinto beans and peas.

Table-1: Nutritional composition of cooked pinto beans and peas – value per 100g of product (Adapted from 18)

Nutrient	Unit	Pinto Beans	Peas
Proximate composition			
Water	g	62.95	77.87
Energy	kcal	143	84
Protein	g	9.01	5.36
Total lipid (fat)	g	0.65	0.22
Carbohydrate, by difference	g	26.22	15.63
Fiber, total dietary	g	9.0	5.5
Sugars, total	g	0.34 5	93
Minerals			
Calcium, Ca	mg	46	27
Iron, Fe	mg	2.09	1.54
Magnesium, Mg	mg	50	39
Phosphorus, P	mg	147	117
Potassium, K	mg	436	271
Sodium, Na	mg	1	3
Zinc, Zn	mg	0.98	1.19
Vitamins			
Vitamin C, total ascorbic acid	mg	0.8	14.2
Thiamin	mg	0.193	0.259
Riboflavin	mg	0.318	0.149
Niacin	mg	0.229	2.021
Vitamin B-6	mg	0.054	0.216
Folate, DFE	µg	172	63
Vitamin B-12	µg	0	0
Vitamin A, RAE	µg	0	40
Vitamin A, IU	IU	0	801
Vitamin E (alpha- tocopherol)	mg	0.94	0.14
Vitamin D (D2+D3)	µg	0	0
Vitamin D	IU	0	0
Vitamin K (phylloquinone)	µg	0.35	25.9
Lipids			
Fatty acids, total saturated	g	0.136	0.039
Fatty acids, total monounsaturated	g	0.133	0.019
Fatty acids, total polyunsaturated	g	0.235	0.102
Cholesterol	mg	0	0

Food and feed uses of legumes

Legumes play major roles in traditional recipes in many regions of the world and are the major source of protein intake in developing countries [19]. Legume seeds can be processed into flour and used as main ingredient in sweets, desserts and savoury products [20]. One of the most frequently consumed legumes in the world is soybean. Soybean is currently processed into different products such as soymilk, soymilk powder, soycheese, tofu, soy sauce, soy flour, soybean oil and tempeh [21]. Legume seeds can be used as a component of rational nourishment and food for vegetarians due to their composition as well as meat replacer. The isolated proteins, starch and fibers from legume seeds have good physicochemical and health properties which promote their application industrially [22, 23]. Legumes are valuable source of feed for livestock [24]. Mature dried legumes seeds are used as feed components for non-ruminant and ruminant animals, majorly because of their high crude protein and amino acid content [25-27]. According to Blair [24], an estimated 20 million tonnes of protein feeds are annually used in compound feeds for livestock. Thus, the use of grain legumes as an alternative protein sources was employed as they supply an important source of plant protein [28]. However, the use of grain legumes in animal nutrition has been hindered due to relatively high concentrations of secondary plant metabolites or antinutritional factors (such as tannins, protease inhibitors, alkaloids, lectins, pyrimidine glycosides and saponins) in legumes. These antinutritional factors can cause feed refusals, reduced nutrient digestibilities or toxic effects [29, 30].

Legume phenolics and their bioactivities

Phenolic compounds are part of the important group of secondary metabolites produced by plants. They are characterized by at least one aromatic ring (C6) that bears one or more hydroxyl groups [31]. Phenolic compounds act as radical scavengers, reducing agents, and chelators of metal ions [32]. The dominant phenolic compounds present in leguminous seeds are flavonoids, phenolic acids, and procyanidins [33].

Flavonoids are the predominant components in legumes [34, 35] and flavonoid extracts of legumes demonstrated antioxidant activities [35, 36]. Many flavonoid compounds, such as anthocyanins, quercetin glycosides, and proanthocyanidins (condensed tannins) were reported to be present in the seed coats of common beans [34]. Among flavonoids, isoflavones are the most abundant subclass in legumes [37]. Genistein and daidzein are the reported isoflavones which are found in legumes such as soy beans and chickpeas. Soybeans have significantly high levels of daidzein and genistein, approximately accounting for 47 mg and 74 mg per 100 g soybeans respectively. Chickpeas contain approximately 0.04 mg daidzein and 0.06 mg genistein per 100 g. Isoflavone compounds possess a certain antioxidant capacity, but are relatively weak compared to other polyphenols [38].

Pro-anthocyanidins, also known as condensed tannins, are another class of phenolic substances that have also been identified in some legume seeds such as beach beans [39].

Table-2: Phenolic compounds of some legumes

Legumes	Phenolic acids	Flavonoids	References
Black turtle bean (*Phaseolus vulgaris* L.)	Gallic acid, Ferulic acid, Sinapic acid, Syringic acid,	Myricetin, Catechin, Epicatechin, Quercetin-3-o-glucoside, Kaempferol-3-o-rutinoside, Kaempferol-3-o-glucoside	40
Black soybean (*Glycine max* L.)	Gallic acid, Protocatechuic acid, Vanillic acid, Syringic acid, p-Hydroxybenzoic acid	Catechin, Epicatechin, Quercetin-3-o-glucoside,	40
chickpea (*Cicer arietinum*)	p-Hydroxybenzoic acid, Syringic acid, Gentisic acid	Luteolin-8-C-glucoside, Myricetin-3-O-rhamnoside, Quercetin-3-O-galactoside, Quercetin-3-O-rhamnoside	41
field pea (*Pisum sativum* L.)	Protocatechuic acid, p-Hydroxybenzoic acid	Luteolin-6-C-glucoside, Apigenin-8-C-glucoside, Luteolin-3,7-di-O-glucoside, Apigenin-6-C-glucoside	41
faba bean (*Vicia faba* L. var. minor)	Gallic acid, p-Hydroxybenzoic acid	Epicatechin, Luteolin-8-C-glucoside, Luteolin-6-C-glucoside, Apigenin-8-C-glucoside, Myricetin-3-O-rhamnoside, Apigenin-7-O-neohesperoside	41
white lupin (*Lupinus albus* L.)	Protocatechuic Acid	Apigenin	41
yellow lupin (L. *luteus* L.)	Protocatechuic Acid	Apigenin	41

The *in vitro* antioxidant activity of extracts from common legumes such as pinto bean, cowpea, baby lima bean, lentil, chickpea, small red bean, red kidney bean, black kidney bean, navy bean, and mung bean extracts were investigated by Zhao *et al*. [4]. They reported significant differences in the phenolic content and the antioxidant activity amongst the legume extracts. Lentils showed the highest phenolic content, total antioxidant activity, DPPH scavenging activity, and total reducing power, whereas baby lima bean and navy bean showed the least value. Significant positive correlation was observed between phenolics and total antioxidant activity, DPPH scavenging activity, total reducing power of the different legume extracts.

Phenolics content and antioxidant and anti-inflammatory activities of legume fractions were reported by Boudjou *et al*. [42]. Their work investigated the phenolic compounds from traditional faba beans and lentils grown in Algeria, as well as their fractions (cotyledons and hulls) and potential beneficial effects on antioxidant and anti-inflammatory activities. These fractions, together with their corresponding whole seeds, were extracted with two solvents, aqueous (70%) acetone and (80%) ethanol, and evaluated for antioxidant activity in relation to their phenolic contents. Acetone selectively extracted tannins from faba beans. The hulls always exhibited high antioxidant activity, measured using the reducing power (RP), antiradical activity (DPPH) or oxygen radical absorbance capacity (ORAC) assays. Aqueous ethanol (80%) extract of lentil hulls exhibited high antioxidant and anti-inflammatory activities preferentially inhibiting 15-LOX (IC_{50}, 55 µg/ml), with moderate COX-1 (IC_{50}, 66 µg/ml) and COX-2 (IC_{50}, 119 µg/ml) inhibitory effects on the COX pathway, whereas faba bean hull extracts exerted relatively mild LOX inhibitory activity.

Marathe *et al*. [43] Worked on the comparative study on antioxidant activity of different varieties of commonly consumed legumes in India. They screened thirty different varieties of commonly consumed legumes in India for phenolic content and antioxidant activity using, radical scavenging [(1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) and 2,20-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid, ($ABTS^{\cdot+}$)], Ferric Reducing Antioxidant Power (FRAP) and metal ion (Fe^{2+}) chelation assays. They reported that legumes investigated contained varied amounts of polyphenols and possessed wide range of antioxidant activity. Cowpea (brown and red), horse gram, common beans (black, red, brown and beige), soybean and fenugreek showed excellent antioxidant activity. While, chickpea (cream, green and big- brown), pea (white and green) and lablab bean (cream and white), showed very weak antioxidant potential. Thus, most of the varieties having light colour seed coat, except soybean exhibited low antioxidant activity. While legumes having dark colour seed coat do not always possess high antioxidant activity (e.g. moth bean, black pea, black gram, lentils).

Beninger and Hosfield [34] reported that the seed coats of the common beans contained many flavonoid compounds and they had significant antioxidant activity relative to butylated hydroxytoluene (BHT), a commercial phenolic antioxidant that is often added to foods to preserve fats. Flavonoids in legumes were reported to show improve endothelial function and inhibit platelet aggregation in humans [44]. The antioxidant effect of flavonoids may counteract oxidative stress-induced endothelial dysfunction and platelet aggregation, which are two key causes of cardiovascular disease [44]. Genistein and daidzein are the isoflavones found in legumes such as soy beans and chickpeas. In vivo, daidzein metabolized to equol and odesmethylangolensin, which increase antioxidant activities tenfold compared to daidzein or genistein [38]. Genistein and other isoflavonoids in legumes demonstrate inhibitory effect on LDL oxidation [38, 45, 46]. Ox-LDL is known to stimulate atherosclerotic plaque formation [47, 48]. Proanthocyanidins, also known as condensed tannins which are another class of phenolic substances that have been identified in some legume seeds such as beach beans are capable of scavenging DPPH free radicals in vivo [39, 49]. Proanthocyanidins have been indicated to play a role in cardiovascular diseases via vessel relaxation and LDL oxidation inhibition. Dark coloured legumes such as lentils and black soybeans are reported to possess higher phenolic content and antioxidant activities than their pale colour legume. Dark colour (red, bronze, and black) legumes, such as lentils, coloured beans, and black soybeans had a significantly higher phenolic content and antioxidant capacity than that of the pale colour (green, yellow, and white) legumes, such as yellow peas, green peas, and yellow soybeans [50]. Dark green coloured beach bean contained higher amounts of tannins than their counterparts [51]. In vitro, extracts of black beans, black soybeans, lentils and red kidney beans are more effective in inhibiting LDL oxidation than yellow soybeans [52, 50].

Effect of processing on phenolic content of legumes and their bioavailability

Legumes are usually consumed in processed form. Food processing helps to improve flavor and palatability of food materials and further increases the bioavailability of nutrients, by inactivating antinutritional factors, growth inhibitors and haemagglutinins [53]. Various form of processing methods may be employed. Cooking which is a major form of processing method brings about a number of changes in physical characteristics and chemical compositions of dry legumes. Pressure boiling and steaming can also be used. Prior to cooking, soaking is a preliminary step, it helps soften texture and shorten the cooking time. High pressure processing technology may provide high quality of food products (flavor, color, biological active components) [54]. Xu and Chang [55] worked on the effects of soaking, boiling and steaming processes on the total phenolic components and antioxidant activity in commonly consumed cool season food legumes. Green pea, yellow pea, chickpea and lentil were the legumes they investigated. Their work showed that soaking, boiling, steaming processes significantly affected the total phenolic contents and antioxidant activities in all legumes investigated. The changes depended on the type of legume and processing conditions. Steaming processes was reported to cause smaller losses in total phenolic content, antioxidant activities and solid mass than the boiling processes. Due to the ability of the steaming to preserve the antioxidant components and also for decreasing cooking time, they therefore recommended steaming for legumes preparation in domestic and industrial processes. The changes in the overall antioxidant properties of the processed legume was suggested to be due to the synergistic combinations or counteracting of several types of factors, including oxidative reaction, leaching of water-soluble antioxidant compositions, formation or breakdown of antioxidant compositions, and solid losses during processing.

The antioxidative properties and total phenolic contents of raw and processed (dry heating, autoclaving and soaking followed by autoclaving) seed extracts of *Cicer arietinum* and *Pisum sativum* were analyzed by Nithiyanantham *et al*. [56]. The raw and processed seed samples were extracted with 80% methanol and 70% acetone separately and used for the evaluation of its antioxidant potential. Generally, their work revealed that the raw seed extracts were the most potent antioxidant suppliers and free radical scavengers. Moreso, the processing methods, such as dry heating, autoclaving and soaking followed by autoclaving, significantly affected the total phenolic contents and antioxidant activities in all samples. The changes depended on the type of legume and processing conditions. Dry heat processes caused smaller losses in total phenolic content and antioxidant activity. Their work therefore suggested that dry heating processing method proved to be advantageous in retaining the integrity of the appearance and texture of the legume with greater retention of antioxidant components and activities.

Chen *et al*. [57] studied the characterization of free, conjugated and bound phenolics and lipophilic antioxidants in regular- and non-darkening cranberry beans (*Phaseolus vulgaris* L.). Seven different cultivars of cranberry beans were characterized for phytochemicals and assessed for antioxidant activities. Total phenolic (TPC) and total proanthocyanidin (PAC) contents were measured while free, conjugated and bound phenolic acids and flavonoids were identified and quantified. Regular-darkening (RD) seeds contained higher TPC, PAC and flavonoids which were absent in the non-darkening (ND) seeds. Bound and conjugated phenolics in RD and ND mainly included cinnamic and benzoic acids. DPPH, FRAP and ORAC showed strong positive correlation with TPC, PAC, and with specific phenolics such as free catechin and bound p-hydroxybenzoic acid. Lipophilic extracts were rich in polyunsaturated fatty acids (69.20– 76.89%). Carotenoid and tocopherol were limited to γ-tocopherol and β-carotene.

Bioavailability can be defined as that fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites where it can exert its biological action [58]. It was originally used in pharmacology to define the concept of the "rate and extent to which a drug reaches its site of action". Bioavailability of legumes phenolic compounds simply means how much of the ingested quantity of the phenolics that is able to exert its beneficial effects in target tissues.

Epidemiological studies have suggested associations between the consumption of polyphenol-rich foods or beverages and the prevention of diseases. Cancer can be prevented through the consumption of tea, fruit and vegetable and also prevent stroke [59], whereas wine consumption might prevent coronary heart disease [60]. Polyphenols are reducing agents, and together with other dietary reducing agents, such as vitamin C, vitamin E and carotenoids. They protect the body's tissues against oxidative stress. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others. Phenolic acids are abundant in foods. The most frequently encountered are caffeic acid and, to a lesser extent, ferulic acid. Ferulic acid is associated with dietary fiber and is linked through ester bonds to hemicelluloses.

To establish the evidence for the effectiveness of polyphenols in disease prevention and human health improvement, it is necessary to know the bioavailability of polyphenols [58]. The polyphenols that are the most common in the human diet are not necessarily the most active within the body, either because they have a lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated. Furthermore, the metabolites that are found in blood and target organs and that result from digestive or hepatic activity may differ from the native substances in terms of biological activity [61].

Metabolism of polyphenols occurs via a common pathway [62]. The aglycones can be absorbed from the small intestine, but most polyphenols present in food are in the form of esters, glycosides, or polymers which cannot be absorbed in their native form. These substances must be hydrolyzed by intestinal enzymes or by the colonic microflora before they can be absorbed. The efficiency of absorption is often reduced when the flora is involved, because the flora also degrades the aglycones that it releases and produces various simple aromatic acids in the process. During the course of absorption, polyphenols are conjugated in the small intestine and later in the liver. This process mainly includes methylation, sulfation, and glucuronidation. This is a metabolic detoxication process common to many xenobiotics that restricts their potential toxic effects and facilitates their biliary and urinary elimination by increasing their hydrophilicity. The conjugation mechanisms are highly efficient, and aglycones are generally either absent in blood or present in low concentrations after consumption of nutritional doses. Polyphenols and their derivatives are eliminated chiefly in urine and bile. Polyphenols are secreted via the biliary route into the duodenum, where they are subjected to the action of bacterial enzymes, especially β-glucuronidase, in the distal segments of the intestine, after which they may be reabsorbed [61].

The best method for bioavailability studies is through controlled human subjects, but these studies are laborious and resource intensive, which limit their use to a few number of samples. Also, ethical issues are the major constraint in the use of humans and other animals for trials. Simple, inexpensive, rapid and reproducible in vitro methods have been developed for initial screening of bioavailability [63].

Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated in vitro gastrointestinal digestion was reported by Nderitu *et al*. [64]. In their study, they determined the effect of simulated gastrointestinal digestion on phenolic composition of cooked cowpeas and the ability of the digests to inhibit radical-induced DNA damage. A red and a cream-coloured cowpea type were used. The phenolic composition of the extracts and enzyme digests of cooked cowpeas was determined using UPLC-MS. The results of their study indicated that simulated gastrointestinal digestion of cooked cowpeas affected their phenolic composition. The enzyme digest of the red cowpea type was about thrice as effective as that of the cream cowpea type in protecting DNA from oxidative damage. The observation that enzyme digests of cooked cowpeas inhibited radical induced DNA damage suggests that cowpea phenolics retain some radical scavenging activity after gastrointestinal digestion.

The health effects of polyphenols depend on both their respective intakes and their bioavailability, which can vary greatly.

Health benefits of legume

Epidemiological studies have shown that the consumption of phenolic-rich foods such as legumes is inversely correlated with the prevalence of cardiovascular diseases [65] and type-II diabetes [66]. Polyphenols contained in these food materials have been reported to possess many health promoting and disease-preventing properties [67]. Diabetes for instance, is partly caused by the excessive presence of carbohydrates in the diet and starch digestion in mammals is mainly carried out by α -amylase and α-glucosidase [68]. Inhibition of starch digestive enzymes or glucose transporters can suppress postprandial hyperglycemia by reducing the rate of glucose release and absorption in the small intestine [69]. Phenolics in legumes can inactivate α -amylase, α -glucosidase and lipase through non-specific binding to enzymes [70].

Tan *et al*. [40] worked on the Comparison of α-amylase, α -glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera namely black soybean (Glycine max) and black turtle bean (Phaseolus vulgaris). Their results showed that out of all the five eluted fractions, fraction V from black soybean was the most effective (IC_{50}: 0.25 mg/mL) against α -amylase; Fraction V from black turtle bean was the most potent (IC_{50}: 0.25 µg/mL) against α -glucosidase; Fraction IV from black turtle bean was the most powerful (IC_{50}: 76 µg/mL) against lipase. Out of the pure phenolic compounds tested, myricetin showed the highest inhibition of α -amylase, α -glucosidase and lipase (IC_{50}: 0.38 mg/mL, 0.87µg/mL and 15 µg/mL, respectively). Managing postprandial plasma glucose levels is important in the early treatment of diabetes. Inhibiting enzymes like α -amylase and α -glucosidase, which are involved in the carbohydrate digestion, is an important method for decreasing postprandial hyperglycemia [71]. Since the fractions from the two black legumes they investigated were able to inhibit α -amylase, α -glucosidase and lipase, their study therefore contributes to the understanding of the potential effectiveness in the use of the two black legumes for the management of diabetes [40].

CONCLUSIONS

Legumes are nutrient dense food crop used as staple food in developing countries and other regions of the world. The nutrient composition, phenolic and bioactivities of legume phenolics depend on legumes type. A number of studies confirmed the potentials of legumes phenolics in reducing the risk of cardiovascular diseases and type-II diabetes. Processing methods such as soaking, boiling and steaming can results in losses in phenolic contents and reduce antioxidant activities of legumes. Although legume proteins are good alternative protein for livestock feed, the toxicological risks due to antinutritional factor could be reduced by processing the legumes prior to their incorporation into animal feeds.

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