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Edited by Jose Carlos Jimenez-Lopez

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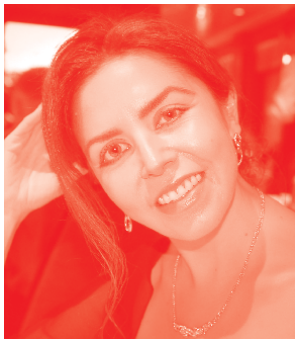

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Meet the editor

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Contents

Preface

Legumes are a treasured and cost-effective source of high-quality proteins (20–50% of seed content) in the human diet, with outstanding nutritional and nutraceutical properties. They jointly encompass the necessary genetic diversity to cope with different environmental stresses that are becoming more numerous while stimulating the agriculture and food security industries as climate resilience effects are increasingly accentuated. In the near future, humanity will have to face many global challenges, a good number of them are driven by climate change. These include global food security strategies to develop sustainable protein sources for an increasing population, reducing waste production, lowering the release of greenhouse gases, and promoting recycling and a more circular economy. In this context, legumes are fundamental crops meeting the needs as significant sources of plant-based proteins—for humans and livestock and at an affordable cost.

Legume proteins play a key role in food nutrition. However, more recently, the nutraceutical aspects of a variety of legume seed compounds are being investigated for their numerous health benefits. These compounds contain anti-inflammatory molecules with properties that assist in the treatment and/or prevention of cardiovascular diseases, type II diabetes, obesity, metabolic syndrome, cancer, etc. Specifically, these are storage proteins belonging to proteins of different families, such as Vicilin (7-S type globulin), Legumin (11S-type globulin), 2S albumin, glutelins, enzymes, enzyme inhibitors, and lectins, which integrate part of the defensive mechanism of the seed. These positive properties of legume proteins on human health may be a consequence of unique structure-functional features, raised properties of derived peptides from their hydrolysis, or even modification of legume proteins for improved functionality and digestibility.

Legume proteins from pea, lentil, lupine, chickpea, and other types of beans are valuable for functional food production, making them outstanding ingredients for food production with improved nutritional and technological properties; they show a wide range of techno-functional properties such as emulsification and stability activity, foam formation and stabilization, gel formation, and water-holding capacity. These technological characteristics are fundamental for the ultimate effect that food containing legume proteins will have on human health. Knowing the advantages and potential inconveniences of legume seed proteins in food production, industrial applications can be more easily achieved.

This book provides an overview of the health benefits, functional properties, and industrial applications of legume seed compounds, mainly proteins. The book also includes updated information from original research and providing novel research literature data on these topics.

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Section 1

Nutricional and Nutraceutical Composition

Chapter 1

Current Advances Research in Nutraceutical Compounds of Legumes, Pseudocereals and Cereals

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Abstract

The increase of the Western-type diet and life-style, with high content of highly processed fats, salt and sugar, as well as sedentary life, is directly linked to an increasing incidence of chronic diseases such as diabetes and obesity, cancer, cardiovascular diseases or stroke, and inflammatory-related diseases, which are a great challenge in global health and are usually associated with negative effects of globalization: rapid urbanization, diet and increased sedentary life worldwide. This has brought new interest and increased research into plant-based diets. In this context, the implementation in the diet of legumes, cereals and pseudo-cereals, due to their nutraceutical properties, which is interesting as well as advisable. These foods, in addition of having a high nutritional value themselves, have synergistic properties as part of a balanced diet. For example, most legumes are rich in lysine which is scarce in cereals, and these are rich in sulphur amino acids, such as methionine, while these amino acids are scarce in legumes and are of great importance for the central nervous system development. These foods or part of a food, due to their qualities, and that they provide health benefits can be classified as nutraceuticals. In addition, due to their health benefits beyond nutritional properties, can be classified as functional foods, promoting prevention and treatment for the above mentioned diseases, among others. This double function is due mainly to the proteins and the presence of various secondary metabolites and bioactive compounds in these foods of plant (grain and seed) origin. Last discovered knowledge and research features will be described in the present book chapter.

Keywords: functional food, nutraceutical benefits, legumes, pseudo-cereals, anti-inflammatory properties, cancer, diabetes mellitus

1. Introduction

Legumes are a main source of edible seeds and a major source of food for a significant worldwide population. They are a relevant sources of plant rich quality proteins (20–50% of seed content) with nutraceutical and health benefit properties

on human health helping to prevent diseases such as diabetes, digestive tract diseases, cardiovascular diseases, overweight, obesity, cancer, etc. Legume seeds are also integrated of fiber, carbohydrates, amino acids, micronutrients as several vitamins and minerals. Anti-nutritional compounds have been found in legumes, which may be toxic when consumed raw, while playing a positive role when processed and treated. Despite of the wide germplasm of legume, there are many underutilized food legume seeds with potential to be a source of nutraceutical food [1].

On the other hand, cereals are essential foods providing key nutrients to many countries worldwide. They are frequently consumed removing a large fraction of the whole gain (40%, mainly bran and germ), however and despite of this fact, wheat-based processed foods contains a large part of components with health benefits such as phytosterols/stanols, carotenoids, polyphenols, dietary fibers such as β-glucan and arabinoxylan, carbohydrates such as resistant starch and oligosaccharides (galacto- and fructo-oligosaccharides), vitamins, selenium and folate. They contain phytoestrogens and antioxidants required molecules promoting health benefits. Cereals are also implemented as fermentable substrates promoting probiotic microorganisms' growth. These macromolecules contribute to reduce the risk of major chronic diseases in humans, such as cardiovascular diseases, Parkinson's disease, as well as cancer risk, reduces the rate of cholesterol and fat absorption, lowering blood pressure, and gastrointestinal health. Among the factors affecting the bioactive macromolecules content of cereal as important food ingredients, it can be included the genetics, growing and storage conditions, post-harvest treatments, food formulation and processing.

Therefore, pseudocereals grains, belonging to dicotyledonous plant species, are growing in interest for human diets because their excellent nutritional and nutraceutical value. Pseudocereals are a good source of fiber, proteins, starch, minerals, vitamins, and phytochemicals such as saponins, polyphenols, phytosterols, phytosteroids, and betalains with potential health benefits [2].

Pseudocereals are considered very healthy grains because their content in several bioactive components with high nutraceutical potential. Among these, it encompasses essential amino acids mainly arginine, methionine, lysine, tryptophan, and sulphur-containing amino acids in large amounts. They are also source of polyphenols, saponins, fagopyritols, phytosterols, and essential minerals. However, pseudocereals contain anti-nutrients like phytates and tannins, which can be decreased by processing treatments as soaking, puffing, germination, fermentation, and cooking to also improve their organoleptic characteristics. These treatments improve the nutritional value of pseudocereals by decreasing anti-nutrients amount, while increasing the availability of nutrients. An advantage of pseudocereals is their uses in food for persons suffering from celiac disease due to the absence of gluten in their grains, and in general its great potential to be utilized for the development of functional foods [1, 2].

This chapter discuss current research advances in nutraceutical properties and key research knowledge on health-promoting aspects of seed bioactive components found in legumes, cereals and pseudocereals, for increasing awareness among the population of the research focus across the beneficial health effects, and disease prevention activities.

2. Legumes

2.1 Antioxidants and anti-inflammatory compounds

Diseases such as obesity, cardiovascular problems, diabetes, inflammation and cancer are related to oxidative stress (OS). When cellular systems are not capable of

efficiently eliminating the reactive species produced of oxygen (ROS) and nitrogen (RNS). These produce alterations throughout oxidative processes in different biomolecules such as proteins, lipids and nucleic acids. This can lead to cell death or produce mutations at the DNA level that contribute to the generation of cancer [3].

Legumes, especially the seed coat, are rich in antioxidant compounds. These can terminate oxidative reaction chains by eliminating free radicals and the inhibition of other oxidative reactions [4]. Among the antioxidant compounds present in legumes, flavonoids should be highlighted. The antioxidant capacity of flavonoids is due to the large number of hydroxyl substitutions, which has a direct effect on their ability to donate hydrogen atoms and eliminate free radicals from the environment [5].

Recently, it has been found that conglutins β1, β3 and β6, main types of reserve proteins in *Lupinus angustifolius* seeds, present antioxidant activity, among other health benefits [6], are capable of reducing the expression of pro-inflammatory cytokines IL- 1β, IL-2, IL-6, IL-8, IL-12, IL-17, TNF-α and IFNγ, in addition to decreasing the levels of NF-κB, NO and iNOS, which can make its consumption adequate in the treatment of certain pathologies. In pancreatic cells, these conglutins also reduce the expression levels of chemotactic factors as CCR2 and CCL5, improving the inflammatory state by inhibiting the recruitment and migration of immune cells [7].

2.2 Cancer fighting seeds components

As part of the cancer development, progression, and metastasis, proteolysis process plays an important role. Proteolysis is involved in the degradation of the extracellular matrix causing changes in cellular adhesion, migration, invasion, as well as chemical modification of the cellular environment, including the production of growth factors [4]. Protease inhibitors (PIs) present in the protein fraction of seeds of certain beans as *Phaseolus acutifolius* affect the proliferation and metastasis in fibroblasts, and also affect cell survival and proliferation, inducing partially restoration of the adhesion patterns of the transformed fibroblasts. Other PIs from *Phaseolus vulgaris* seeds inhibit the proliferation of MCF-7 breast cancer cells showing a slight inhibition of the proliferation of hepatoma HepG2 cells and WRL68 embryonic liver cells [4].

Therefore, ROS and RNS generated during carcinogenesis modify gene expression, regulate signals of transduction pathways and modulate protein function, while promoting the activation of enzymes related to angiogenesis [3]. Phytic acid, present in legume seeds exhibits antioxidant function that can regulate proliferation and apoptosis. These processes can be identified by changes in biomarkers of colon cancer cells, suppressing the expression and activity of key regulatory factors of the AKT/mTOR pathway, AKT1 kinase and p70S6K1 [3].

Therefore, it has been found that the main flavonoids present in *P. vulgaris*, quercetin and kaempferol are able to decrease the risk of lung cancer [8]. Quercetin-3-O-glucoside, present in black beans, also reduces the expression of lipogenic proteins helping to improve cardiovascular disease [5]. On the other hand, the flavonoid genistein inhibits carcinogenic cells, being useful against breast and prostate cancers [8].

Extraction samples with ethanol from *Phaseolus angularis* seeds can inhibit the release of prostaglandin E2 and nitric oxide in macrophages induced by lipopolysaccharides (LPS) in a dose-dependent manner, with inhibition of the nuclear factor NF-κB and response of the activating protein AP-1 [4]. Extracts obtained from *Phaseolus calcaratus* prevent nitric oxide production and release, iNOS and COX-2 genes expression, and TNF-α and IL-6 proteins secretion almost totally

in LPS-stimulated cells. Furthermore, phenolic compounds with antioxidant potential against DPPH and hydroxyl radicals exhibit anti-inflammatory potential in LPS-stimulated macrophages through down-regulation of ERK/p38 and NF-κB-mediated signalling pathways [4].

2.3 Metabolic syndrome: diabetes, obesity and cardiovascular diseases

Patients diagnosed with diabetes, persistent hyperglycaemia increases the generation of free radicals, which initiates lipid peroxidation and proteins oxidation, altering the structure of the membrane, which in turn leads and promote other complications such as insulin resistance [9].

Phytosterol β-sitosterol, rich among legume lipids, has antioxidant activity and acts as a ROS scavenger facilitating the membrane stabilization. Rats with induced type 2 diabetes showed decrease in the levels of insulin receptors (IR) and glucose transporters GLUT-4. In addition, administration of β-sitosterol resulted in a restoration of the levels of these above membrane proteins, which improved glycaemic control [9]. β-Sitosterol is structurally similar to cholesterol, thus its administration allowed inhibition of intestinal cholesterol absorption while acting as an antioxidant and chemopreventive potential for the appearance of colon cancer [9].

The hydroethanolic extract of *Lupinus mutabilis* seeds, rich in sparteine, palmitic acid, linoleic acid, oleic acid, lupanin, oxilupanin and 11,12-dehydrolupanin, has also been observed to have positive effects in rats with type 2 diabetes by enhancing insulin release. These effect is dependent on the L-type calcium channel, protein kinase A and C systems, and G protein-coupled exocytosis and is partially mediated by K-ATP channels [10].

Soybeans contains isoflavonoids with estrogen-like activity because they have agonist and antagonist receptors activity. Since estrogen has antidiabetic effects by increasing insulin secretion, decreasing insulin resistance and increasing pancreatic β cell mass, these isoflavonoids could have positive effects in individuals with type 2 diabetes [11].

Protease inhibitors regulate the hydrolytic action of proteolytic enzymes. The balance action between proteases and PI is required for a suitable cellular homeostasis, thus when unbalanced, pathological progressions such as cancer are likely to develop [4]. Inhibitors of carbohydrate hydrolases such as α-amylase and α-glucosidases, present in the seeds of numerous legumes, represent a route to lower glucose levels after meals (postprandial glycaemia), both in patients with type 1 and type 2 diabetes. 2 [4, 12]. α-Amylase acts on the α-1,4 glycosidic bonds of starch, which is one of the main sources of postprandial glucose, while intestinal α-glucosidases act on different oligomers of glucose. The peptides present in legume seeds inhibit the degradation of these carbohydrates by shifting the binding site of the substrate in the enzyme and shifting it from the active center [12]. Furthermore, bioactive peptides have been found in soybean germinated seeds that have inhibitory activity of dipeptidyl peptidase 4 (DPP-IV), a serine protease capable of degrading incretins, gastric inhibitory polypeptide (GIP) and glucagon-like peptide type 1 (GLP-1). These incretins are secreted after meals and stimulate insulin secretion, thus the protease inhibitors of these incretins present in soy represent another treatment for patients with diabetes [12]. On the other hand, legumes are a rich source of amino acids, among which arginine stands out. Arginine has proven to be useful in reducing insulin resistance, which is why the intake of legumes is thus equally recommended in diabetic individuals [8]. In this regard, L-homoarginine, especially notable in *Lathyrus sativus* L. provides benefits in individuals with cardiovascular diseases [1].

Interestingly, *Lupinus angustifolius* seed conglutins β1, β3, and β6 are capable of acting positively against diabetes [13]. This activity is mediated by its antioxidant and regulatory activities by (i) increased cellular glucose uptake, (ii) positive regulation of IRS-1, GLUT-4 and increased protein synthesis of p85-PI3K, (iii) activation of IRS-Q/PI-3-KINASE, which activates other components of the signalling cascade, (iv) reduction of oxidative stress reducing the level of protein carbonylation and increasing glutathione levels GSH and decreasing the antioxidant activity of SOD and catalase enzymes, (v) reduction in NO production levels, (vi) increased glucose catabolism, increasing the expression of, among others, hexokinase and decreasing that of GSK3β [6, 14]. Furthermore, the already described anti-inflammatory activity of these conglutins β could also help in the treatment of diabetes by reducing the inflammation of the pancreatic islets [7]. In the same way, it has been found that γ conglutins of the same species have similar activities [15].

Bioactive peptides such as Lunasin, a 43 amino acid peptide, is found in soybeans, barley, rice and wheat, and is capable of lowering cholesterol levels and increasing levels of LDL receptor production, as well as exhibiting anti-inflammatory properties. Interestingly, lupine conglutins exhibit similar cardiovascular protective activities by decreasing LDL levels and increasing their receptors in hepatoma lines in rats [16].

2.4 Anti-nematode agents

Gastrointestinal infections by nematodes are currently a problem due to the development of resistance to anthelmintic drugs [2]. The consumption of legumes, rich in tannins and flavonoids (such as flavanols) has been related to resistance to these infections in ruminants, due to the activity of these compounds against nematodes. Particularly, the proportion of prodelphinidin/procyanidins are related to the anthelmintic activity of legumes, as well as their consumption acting indirectly to the potential of the individual's immune system fighting against parasites [2].

2.5 Health benefits of anti-nutritional compounds of legumes

Despite the large number of health-beneficial compounds present in legumes, their seeds also contain a fair number of anti-nutritional components. These compounds can be inactivated through food preparation [4]. There are also numerous studies using germplasm resources searching for varieties with low amount of these components, and to be implemented in the diet [1]. These anti-nutritional properties usually are visible in non-balanced diets, even in certain concentrations are beneficial.

Lectins proteins or glycoproteins included in their structure include at least one non-catalytic domain with the ability to bind mono and oligosaccharides [4]. Among their negative properties, they can cause atrophy of the pancreas and interfere with the absorption of nutrients by binding to the intestinal epithelium. However, they are recommended in certain disorders, such as diabetes, obesity, and cancer [4]. *P. vulgaris* is rich in the lectin called phytohemagglutinin. Seed extracts have been shown to reduce postprandial blood glucose in rats in a similar way to metformin, the first choice drug for the type 2 diabetes treatment. The mechanism of action involves binding of phytohemagglutinin to epithelial cells gastric cells and the membrane of the cuticular border of the small and large intestine, which causes the secretion of cholecystokinin and glucagon-like peptides, two hormones that play an important role in digestive processes and the central control of appetite [4]. Lectins also have negative effect on the appearance of appetite and promoting secretion of cholecystokinin and glucagon-like peptides. In addition, lectins bind to

membrane or intercellular receptors affecting cell cycle disruption and induction of apoptosis, inhibition of telomerase activity, and inhibiting angiogenesis.

L-Dopa, an amino acid precursor of the neurotransmitters dopamine, norepinephrine, and adrenaline, is present in high quantities in legumes such as *Mucuna pruriens* and *Vicia faba*. This compound is useful in the treatment of Parkinson's [17], as a chronic and progressive process caused by neuronal degeneration in the substantia nigra, leading to a decrease in dopamine values, which in turn decreases the individual's ability to control movements or feelings. L-Dopa would have the property of restoring neurotransmission [17]. Intestinally, L-Dopa and dopamine, both function as prolactin inhibitors (hormone that decrease the sexual desire), thus composition of *Mucuna pruriens* is used in some regions as an aphrodisiac [17].

β-ODAP or β-oxalyldiaminopropionic acid is a glutamate analogue and the cause of neurolathyrism produced after a high intake (more than 30% of the caloric intake) of *Lathyrus sativus* prolonged for a long time. However, a balanced diet could have positive effects in individuals with Alzheimer's [1], since β-ODAP has an activating effect on protein kinase C, which is involved in the memory processes, thus the balanced intake of *Lathyrus sativus* could be recommended in these individuals. On the other hand, β-ODAP has properties as wound healing agent, being useful to tamponade haemorrhages [1].

2.6 Nutritionally fortified food based in legumes

In recent years, research has focused on the development of derivatives of highly consumed products, such as bread or milk, produced by adding legume seed extracts allowing exploiting their nutraceutical potential [18–20].

Bread produced from sprouted lentil extracts added to wheat flour is a rich source of lysine, a scarce amino acid in cereals. Additionally, this added extract increase up to 100% in phenolic acids (quercetin and isorhamnetin) to common bread [18].

Vegetable milk substitutes are an ideal vehicle for the introduction of essential nutrients and nutraceuticals in the human diet because it can be fortified with bioactive ingredients of different plants. In this context, milk substitutes obtained from legumes can be easily fortified with micronutrients such as vitamin B12, vitamin D, calcium and omega-3, scarce in legumes [19].

Mayonnaise preparation replacing the egg yolk with proteins of vegetable origin, such as chickpeas, beans and lupine, would be an easy way to integrate phenolic acids, α-amylases and glucosidases in this food, turning this condiment into a food with antioxidant, antihypertensive and antidiabetic properties [20].

3. Pseudocereals

Pseudocereals have attracted great attention in the last 20 years for their nutritional properties. They content large quantities of fiber high quality protein (that includes essential amino acids rich in sulphur), and starch. They are a good source of iron, zinc and calcium, vitamins, saponins, phytosterols, polyphenols, phytosteroids, and betalains. Pseudocereals have higher content of lysine, methionine and cysteine compared to common cereals, mainly deficient in lysine and secondarily deficient in threonine and tryptophan [21]. The groups of bioactive components in pseudocereal grains include saponins, phenolic compounds, phytosterols, phytoecdysteroids, polysaccharides, betalains, and bioactive proteins and peptides [21].

Saponins are steroid or triterpenoid glycosides predominantly found in seeds. The saponin fraction present in quinoa and including 3-O-β-D-glucopyranosyl oleanolic acid, has been shown to exhibit anti-inflammatory activity in murine macrophages induced by lipopolysaccharides by inhibiting the production of nitric oxide (NO), tumor necrosis factor (TNF)-α, and interleukin IL-6 [22].

Quinoa saponins have also shown *in vitro* antiobesic effects by inhibiting the accumulation of triglycerides in adipocytes and suppressing adipogenesis [21]. Furthermore, it has been observed that they are capable of inhibiting the expression of PPARγ, C/EBPα and SREBP1c, which act as transcription factors during adipocyte differentiation [23].

Saponins have membranolytic activity. The aglycone group of the saponins structure has affinity with the lipid region of the cell membrane, generating disturbance in the fluidity and permeability of the cell membrane. The membranolytic activity of quinoa saponins on cells of the small intestine can cause an increase in the permeability of the mucosa, which would promote an increase in the rate of exfoliation of intestinal cells, which is associated with an increase in the loss of cholesterol by secretion faecal bile acids and neutral steroids, given the ability of saponins to bind to cholesterol these bile acids [23].

Quinoa extract inhibits the formation of the GSSG dimer and favours the GSH form through the activation of Glutathione-S-transferase (GST) by the action of H_2O_2. Thus, the quinoa extract acts as a reducing agent for disulphide bridges [23]. These activities are directly linked to surfactant and antioxidant properties, and were most of the diseases associated to oxidative stress are characterized by a decrease in GSH or the GSH/GSSG ratio.

Phenolic compounds found in Pseudocereal flours have revealed a wide variety content of flavonoids (anthocyanins, isoflavonoids, flavonols, flavones, flavanones, and flavonoids), phenolic acids and derivatives of tyrosol.

In this regard, squalene is an inhibitor of mRNA expression of 3-hydroxy-3-methylglutaryl coenzyme A, a key reductase enzyme in cholesterol biosynthesis [23]. Rutin, a glucoside flavonoid with numerous pharmacological activities present in buckwheat including its seed [24] shows many benefits such as its anticarcinogenic capacity in numerous types of cancer, neuroprotective and cardioprotective activities, including antihypertensive and anticoagulant effects [25]. Cinnamic acids (hydroxycinnamic acid, hydroxybenzoic acid, caffeic acid and chlorogenic acids), coumarins and isocoumarins contains in pseudocereals have shown high antioxidant activity. Chia seeds are abundant in caffeic acid and chlorogenic acid, which antioxidant activity has been proven *in vitro* but not *in vivo* assessment. However, the effect of caffeic acid on health is controversial since its consumption has also been linked to cancer [26].

Anti-aging agents found in pseudocereals have been proposed as promising bioactive molecules with protective effect against skin aging due to their ability to scavenge free radicals, chelate metal ions, and inhibit collagenase activity in the skin [21].

Phytoecdysteroids with antioxidant capacity, have an anti-obesity effect, assuming a significant reduction in fat mass that is attributed to greater carbohydrate oxidation and fecal lipid excretion in rats [21].

Polysaccharides (carbohydrates) made up of galacturonic acid and glucose monosaccharides contained in quinoa seeds exhibit radical scavenging effects, macrophage proliferation-promoting properties, suppression of NO production, and cytotoxic activity against MCF-7 breast cancer cells [21]. In addition, buckwheat polysaccharides facilitate the secretion of several cellular factors, including TNF-α, NO, IL-2, and IL-1β in macrophages, and show potential for the treatment of leukaemia [21]. ***Phagopyritols*** have antioxidant, anti-inflammatory and antidiabetic

activity. Buckwheat phagopyritols significantly suppress increased blood glucose, decreased lipid levels, and improved insulin resistance *in vivo* in an insulin-resistant mouse model. Furthermore, these carbohydrates enhanced glucose uptake in both normal and insulin resistant HepG2 cells [21].

Consumption of ***D-phagemine*** induces a reduction in postprandial blood glucose concentration by inhibiting intestinal disaccharidases. In addition, it achieves the reduction of weight gain, inflammation and impaired glucose tolerance, most probably by influencing the intestinal microbiota. D-phagomin stimulates the diversity of the gut microbiota by increasing bacteroids populations in healthy rats and mitigates the age-related decline in the supposedly beneficial *Lactobacillus* and *Bifidobacterium* populations. In addition, the ability of D-phagemine to counteract sucrose-induced steatosis and hypertension, is due presumably by reducing postprandial hepatic fructose levels [21].

Fiber present in the pseudocereals is also capable of inhibiting the absorption of cholesterol, while binding to bile acids favours the catabolism of cholesterol or the fermentation of fiber in the colon, promoting short-chain fatty acids that contribute to the reduction of cholesterol synthesis in the liver [23].

Bioactive peptides composition in pseudocereals include different families with multiple health benefits. The main mechanism controlling blood pressure is based on the renin-angiotensin-aldosterone system (RAAS) and angiotensin converting enzyme (ACE) inhibitors. ACE is responsible for the conversion of angiotensin I to angiotensin II, which increases peripheral vascular resistance, and inducing a hypertensive action [16]. Pseudocereals as Amaranth contains a large amount of 11S and 7S globulin proteins with antihypertensive effects. The breakdown of these proteins by the enzymes of the digestive tract gives rise to bioactive peptides that have an antihypertensive activity up to 8 times higher than the unmodified proteins and with a similar effect on lowering blood pressure in rats compared to captopril drug [16].

In addition to antihypertensive activity, the 11S globulins of amaranth contain peptides with antioxidant activity, mainly derived from the acid subunit [16]. Other peptides from amaranth with lectin nature has an inhibitory power against malignant cancer cells. Amaranth protein hydrolysate has the ability to rearrange the cell cytoskeleton in certain osteosarcoma lines, inhibiting cell adhesion and inducing apoptosis and necrosis [21]. These protein hydrolysates have immunomodulatory effects on epithelial cells through the NF-ƙB signalling pathways. Particularly, the SSEDIKE peptide has the ability to attenuate the activation of epithelial cells and inhibit the allergic reaction in mice with food allergies, preventing IgE secretion, and controlling intestinal inflammation by preventing NF-ƙs activation [16]. Amaranth peptides derived from globulin and glutelin have the ability to inactivate enzymes associated with type 2 diabetes, such as α-amylase and DPP-IV [16].

Quinoa peptides originated after gastrointestinal digestion show inhibitory activity against DPP-IV, α-amylase and α-glycosylase, being those released during the duodenal phase and presenting the greatest inhibitory effect. Specifically, three peptides derived from 11S globulin with the capacity to inhibit incretin degradation have been identified [27]. Interestingly, it has been observed that protein fraction of quinoa hydrolysed with alkalase has radical scavenging capacity, and this antioxidant activity increases at the same time as the level of digestion.

Seventeen peptides with antioxidant activity have been identified in quinoa derived from 11S globulin and other proteins, some of the including functional motives such as LWREGM, DKDYPK, and DVYSPEAG, IFQEYI and RELGEWGI [28]. Millet, a 14-mer peptide, SDRLLGPNNQYLPK sequence exhibited antioxidant and chelating capacity. Similarly, seven other bioactive peptides have been found in sorghum with antioxidant capacity [29].

On the other hand, lunasin from amaranth is capable of preventing cancer, since it is able to enter the cell nucleus and inhibit the transformation of fibroblast cancer cells [16]. In quinoa, it has been observed that the fraction of peptides below 5 kDa have greater antioxidant capacity, which peptides in the high kDa range exhibited a greater mass anticancer capacity [28].

Antimicrobial peptides around 4 kDa are also present in buckwheat showing capability of inhibition of the reverse transcriptase activity of HIV-1 *in vitro*. In addition, other buckwheat-derived peptides rich in glycine and cysteine exhibited antifungal activity against *Mycosphaerella arachidicola* and *Fusarium oxysporum*. Other peptides found in buckwheat possess antimicrobial activity against gram-positive and gram-negative bacteria [29].

4. Cereals

Cereal consumption can reduce the risk of diseases such as heart disease, type II diabetes, and cancer [30]. The development of nutritious, safe, affordable and sustainable food products to prevent lifestyle-related diseases is important and desirable, thus, foods rich in fiber, which can reduce the risk of non-communicable diseases, have become even more desired for consumers [5, 30].

Cereals are a great source of protein, vitamins, minerals, fiber, and important bioactive compounds. These bioactive compounds are concentrated in the outer layers of the seed, and phenolic compounds (phenolic acids and flavonoids) are among them one of the most important because of their healthy properties [30].

Cereal proteins should be part of a healthy and balanced diet, although they are considered of lower quality compared to proteins from animal products due to their low amino acid profile and low digestibility. The protein content in cereals is determined by genetic and environmental factors, being for example, common wheat (*Triticum aestivum*) a cereal that contain a lower amount of protein than ancient species [30].

Protein quality is determined by the proportion of essential amino acids. The einkorn (old) variety has been shown to have a higher content of essential amino acids (threonine, lysine, valine, methionine, leucine, isoleucine and phenylalanine) compared to modern species, although it is low in lysine and high in glutamic acid [30].

Studying ***the amino acids composition of cereal proteins***, it is possible to find L-theanine that exhibits various health benefits such as anxiolytic and relaxant properties, cognitive and emotional enhancement, neuroprotection, anti-inflammatory, and physiological effects such as lowering blood pressure [31]. Although the presence of this amino acid in the human body is conditioned by its intake, and that L-theanine reacts with free sugars losing its nutraceutical value, it has been determined that the consumption of products wheat-enriched with L-theanine have a beneficial effect on health and well-being, and its enrichment could be carried out by enzymatic modification of wheat flour (gluten trans-amination) [31]. This molecular alteration of wheat flour is used mainly for two purposes: to modify the rheology of the wheat dough, and, conversion of glutamine residues into γ-glutamylamines.

Furthermore, gluten is a very important functional protein, responsible for the viscoelastic property of wheat doughs. It is constituted by two type of proteins, gliadin and glutenin [31, 32]. Gliadin and glutenin are insoluble proteins that have a storage function in the seed [30]. Gluten content in ancient wheats is higher than in modern varieties, in addition of having a higher gliadin/glutenin ratio. On the other hand, although the amount of proteins in millet and wheat is similar, millet does

not contain gluten, which is currently important for the diets of people with celiac disease as responsible for the symptoms [30].

Celiac disease is characterized by the loss of absorption villi in the small intestine, which prevents nutrients from being properly absorbed [32]. Thus, peptides rich in proline are not digested and accumulate in the small intestine, which initiates an immune response. These effects are mainly due to the gliadin fractions of gluten, especially α-gliadin and γ -gliadin [30]. Different gluten-free products have been developed based on wheat flours, but problems at a nutritional, organoleptic and technological level persist, in addition to the fact that these foods are generally more expensive [32].

Since the exogenous process that causes celiac disease is well known, a prevention strategy that focuses on reducing gluten toxicity can be carried out as an alternative to a gluten-free diet [32]. Ribeiro et al. [31] have developed a study by which, it is possible to obtain flour rich in L-theanine by transamination of gluten (catalysed by microbial transglutaminase). This transamination would cause the substitution of glutamine by L-theanine in the constituents of gluten rich, which can prevent the immune stimulation that gluten would cause in celiac disease. Alternatively, non-celiac gluten sensitivity is a disorder characterized by intestinal symptoms and extra-intestinal manifestations (headache, fatigue, depression, muscle pain, dermatitis, anaemia, etc.), and its pathogenesis is still not exactly understood, and currently it has been shown that it does not depend solely on gluten, but that high contents of trypsin-amylase inhibitors (ATIs) that can cause gastrointestinal symptoms by stimulating toll-like receptors (TLRs) [30].

Dietary fibers is one of the most important components of cereals, with positive health effects. Dietary fibers are non-starch polysaccharides, such as cellulose, pectin, arabinoxylan, glucan and lignin, which cannot be enzymatically digested in the human digestive tract until reaching the large intestine, where they can be partially digested [33]. Cereals compounds as fiber has shown beneficial for type II diabetes improvement, such in the case of arabinoxylan [33].

Furthermore, beta-glucans are polysaccharides derived from D-glucose molecules linked by beta-glycosidic bonds. It is a part of dietary fibers that are supposed to have a large number of health benefits, including treating some gastrointestinal diseases and supporting the immune system [34]. beta-glucans present in cereals are not digested in the stomach or intestines and have a great ability to form bonds with water, creating sticky gels in the gastrointestinal tract, which results in a delay in gastric evacuation that causes a delay in the action of enzymes that act on starch, obstructing the absorption of digestible carbohydrates. This mechanism causes a reduction in blood glucose as well as insulin secretion, which could contribute to a reduction in the incidence of type II diabetes [34].

Beta-glucans could prevent colorectal cancer, since these act stimulating the immune system by activating macrophages and cytokines production and secretion, among others factors, and binding to immunologically competent cell membrane receptors [34].

Beta-glucans exhibit different properties, such as solubility, degree of branching, and molecular mass and shape, which may have impact on their biological activity [34]. A diet rich in beta-glucans has a positive effect on health by preventing diabetes, hypercholesterolemia, obesity, cardiovascular diseases, and cancer. Beta-glucans are present in cereals as barley or oats, and have been shown to lower blood cholesterol and glucose, in addition of acting as a major factor in the prevention of obesity and metabolic diseases [34]. These present in cereals are not digested in the stomach or intestines and have a great ability to bind water molecules, forming sticky gels in the gastrointestinal tract, which results in a delay in gastric evacuation and a delay in the action of enzymes that act on starch, obstructing the absorption of digestible carbohydrates. This mechanism causes a reduction in blood glucose as

well as insulin secretion, which could contribute to a reduction in the incidence of type II diabetes [34]. They also have a positive effect on lipid metabolism, reducing blood cholesterol (hypocholesterolaemia effect) [34].

In addition, beta-glucans have antioxidant properties by eliminating excess of ROS molecules implicated in diseases. They also have immunostimulatory properties that are beneficial for the prevention of infectious diseases, gastrointestinal cancer, and colorectal cancer, while have prebiotic properties with beneficial effects on the flora of the gastrointestinal tract and prevents diseases of the large intestine and digestive system [34].

Interestingly, recent studies show that the administration of rice bran supplement (RBS) improves sleep *via* histamine H1 receptor (H1R) antagonist. This could represent a breakthrough as a therapeutic agent for insomnia. Furthermore, it has also been observed that H1R antagonists or antihistamines have been used to improve the symptoms of different conditions such as depression, pain, forgetfulness and allergy.

Cereals also contain ***polyphenols*** which are nutraceutical bioactive compounds with antioxidant properties, as well as antimicrobial and immunomodulatory properties [33]. Polyphenols are secondary metabolites synthesized by plants from phenylalanine, and are derived from C6-C3 structures [5]. Ferulic acid is one of the main phenols in wheat. Its content in cereals depends on both the species and the variety, as well as the growing conditions. Phenylpropanoids have beneficial properties for health and those biosynthesized from the aromatic amino acid L-phenylalanine [5]. Its antioxidant capacity is due to the large number of hydroxyl substitutions in each flavonoid molecule, which has an effect on the donor capacity of hydrogen atoms to scavenge free radicals [5]. They are involved in the prevention of cardiovascular diseases, and their metabolism is critical to improve vascular efficiency. The composition of the phenolic compounds is also important, having great effect on the estrogenic activity and the protective efficacy of the flavonoids of *Sorghum bicolour* to prevent colon cancer [5]. Increasing the consumption of fruits and vegetables rich in flavonoids can help control weight, being important with the current global problem of overweight and obesity. Foods high in flavonoids and anthocyanin may be associated with lowering weight gain [5]. Flavonoids improve the function of vitamin C, promoting its absorption and oxidation protection. They also regenerate other antioxidants, such as tocopherols, by donating hydrogen to the tocopheroxyl radical, in a similar way to vitamin C [5].

The bran of cereals is rich in polyphenols, but these are normally eliminated before consumption, and most of the phytochemicals are lost in the development process. Although there is an increase in the consumption of whole cereal (with bran), which is important for polyphenols intake, and therefore their effect, being more effective in terms of health benefits [5].

Therefore, resistant starch (RS) are carbohydrate fractions that persist in the intestine after digestion by pancreatic amylase, reaching the large intestine where it is available for fermentation by the intestinal microbiota [35]. A higher amount of RS in ingested food can prevent or be therapeutic for diseases such as diabetes. RS has different benefits, such as a prebiotic effect, a reduction in the risk of cardiovascular diseases, an improvement in cholesterol metabolism, and a reduction in the risk of colon cancer [35].

5. Conclusion

Legume, cereal and pseudo-cereal seeds are staple food with great importance worldwide for food security, and their seed compounds have demonstrated to be

of high interest at nutritional and nutraceutical level due to large number of health benefits and their potential uses as functional foods.

Many seed compounds have been described and their outstanding properties related to health improvement as anti-inflammatory-related diseases as type 2 diabetes, cardiovascular diseases, cancer, among others. Different molecular mechanisms have demonstrated to be implicated in these health benefits, but many other are still to be studies and deeply understand in order to explore effective diagnosis, therapies, and develop treatment based in grain/seed nutraceutical compounds.

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Conflict of interest statement

The authors have declared that no competing interests exist.

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Chapter 2

Nutraceutical Potential of Seed and Grain Proteins in Health Promotion

Suryapal Singh, Lalita Singh, Harshita Singh and Suman Sangwan

Abstract

In recent years, seed and grain proteins with nutritional bioactivity have been studied for disease prevention and treatments. Seed and grains are key components of a healthy and balanced diet which support the protective role of bioactive proteins with nutraceutical activities. Proteins obtained from seeds can be a good source of amino acids and nutraceutical peptides that can be used for biotic functions to improve health and disease prevention. Hence, the increased consumption of seeds and grains promotes a healthy generation in future and a significant reduction in diseases. To increase the human health awareness, we must have to enlighten the importance of easily available seeds and grains in our food.

Keywords: Seed, grains, proteins, nutraceuticals

1. Introduction

The systematic study of protein grains prolongs back for near about 250 Y, with the separation of wheat gluten first described in 1745 [1]). "TB Osborne" father of plant protein chemistry has been carried out more methodical study of plant proteins in 1859–1929. Cereal grains provide nourishing food globally and food nutritive is essential to all living organisms for function, structure, and regulation of the body. People succeeding an herbal diet need to find no animal sources of protein to ensure they are getting enough nutrients such as Vitamins, Proteins, Minerals and Fibers to prepare their body against diseases. Grains used as protein factories to assist the Nutraceutical and Pharmaceuticals. Seeds and grains enrich in energy as well as factories of essential nutritive fatty acids, flavonoids, bioflavonoids, catechin, epicatechin, quercetin, caffeic acid, coumaric acids, cinnamic acids and many other useful compounds which are used in drug used for the treatment of diseases. Today's generation suffer from infectious, inflammatory, allergic, diabetic, carcinogenic and cardiovascular diseases. Nutraceuticals and drugs are the fastest growing area to cure diseases in humans and other diseases in animals. The production of nutritive elements for nutraceuticals and pharmaceutical applications are very important to save future [2]. Nutraceutical can be well-defined as a food or part of a food that deliver therapeutic and health

aids, with the preclusion and treatment of a disease [3]. Nutraceutical is the combination of "nutrition + pharmaceutical" in broad, are part of food that play a major role in growing and maintaining normal physiological functions that maintains people healthy [4]. Now a day's scientists are focusing on genetic engineering of crops to increase the quantity and quality of protein as a means to produce enormous drugs and vaccines, hoping that this technology can reduce costs and increase the availability of most needed pharmaceuticals and nutraceuticals [5–8]. Seeds and grains-based pharmaceuticals are advantageous because the vaccinated plant tissue can be administered raw, dried, or in an encapsulated form; all forms can be stored and dispatched at room temperature [9]. The risk of contamination with pathogens from animal during production is also removed, [10], moreover seeds-based vaccines can be stored as seeds are beneficial because a lot of vaccines can be produced in very less time and storage is less of an issue because the seed is a stable form that will not degrade the nutritive part for long time. Our main goal through this chapter is to describe the nutraceuticals potential of grain and seed proteins to prevent and treat the disease with an easy and inexpensive way. In India, the week from 24 to 30 July is observed as Protein week to for awareness about the importance of protein in our diet. When the word protein comes to hear, usually muscle or meat comes in our mind but the farmers gold grains are also very important source of proteins. "National Nutrition Monitoring Bureau" Reported in 2017, that consumption of protein in urban Indians was below the recommended dietary allowance, they have taken only 89.8 percent of the recommended amount. In a vegetarian Indian diet, around half of your protein comes from cereals, so this gap could be spanned by including protein-rich diet daily. The comparative protein concentration in seed and grains enlightens the importance and uniqueness of cereals and legumes as nutritive dietary supplements as 3 ounce of chicken contains 20 g protein but 3 ounce of beef contains 21 g of protein, 1 egg contains 6 g of protein, 1 cup of black beans contain 15 g, 2 tablespoons of peanut butter contains 8 g Half a block of tofu contains 18 g protein [11, 24]. When cereals and pulses are combined they can expressively mend the quality of protein in your diet.

2. Applications of seed proteins

There are various types of seeds such as legumes, cereals, vegetables-fruit seeds and oilseeds which enlightens the era of health with vegetarian diet. Seeds are preferred over these systems because they store lots of protein in a relatively small volume and provide a stable environment that promotes protein assemblage and inhibits degradation, thus facilitating long-term storage [8, 11]. For example, antibodies accumulate at high levels in seeds and endure stable for several years with no loss of activity when preserved at room temperatures [12]. In practical terms, this means that cereal seeds containing pharmaceutical nutrients can be stored and disseminated in countries lacking a reliable cold chain. A relatively high protein concentration is achieved because most seeds are small and compact with a simple proteome, which also reduces the number of competing proteins released during processing. Proteins are crucial to all living organisms for function, structure, and regulation of the body. Formation of protein in a cell starts with DNA transcribing into RNA, and RNA translating into proteins. Amino acids (AA) make proteins and are arranged in different combinations and this arrangements and lengths of AA determine the function of the protein, e.g. hormones, enzymes, and antibodies. The given **Figure 1** shows the role of grain proteins in human body in energy production and precursors of other useful molecules.

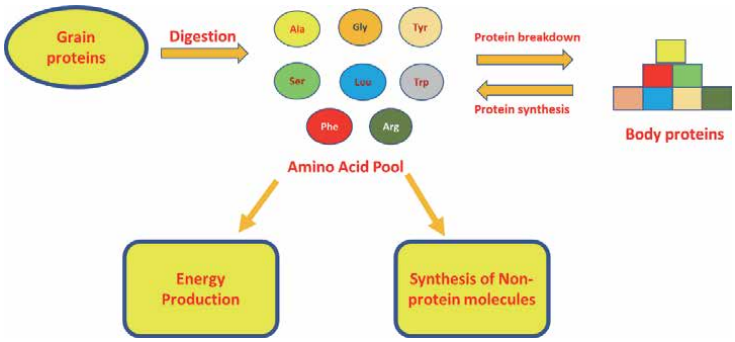

Figure 1.
Role of grain proteins in Human body.

2.1 Legume seeds

The nutritional composition of legume proteins makes it different and useful in food products. Berman et al. [13], in his book chapter focused on the health benefits of legume seeds as potential nutraceutical and also explained the prevention and treatment of certain diseases such as cardiac diseases, diabetes, gastrointestinal infections, obesity, cancer, skin related problems using legumes in diet. Legume seeds also contain resistant proteins that play an active role in human health [14]. Legume seeds contain enzyme inhibitors like alpha-amylase, alpha-glucosidase and gamma-aminobutyric acid (GABA) for which it can be used as a nutraceutical's molecule. Information on legumes grain, presented by FAO (1966), shows consumption to be high in many countries. The popularity of legumes is based on many factors, including their capacity to fix nitrogen to produce a grain containing a high level of protein of a quality which complements the inadequacies of cereal protein. All legume seed proteins are relatively low in sulphur-containing amino acids and tryptophan, but the amounts of another essential amino acid, lysine, are much greater than in cereal grains [15]. Legume seeds such as pea and beans contain 18–20% protein and lupin, soyabean contains 30–35% protein. Variable proteins in legumes shows antifungal, anti-viral, anti-HIV and anti-diabeteic properties, also these proteins are precursors of amino acids which are beneficial to human health [16]. The proteins present in *vigna species*, show anti-fungal and anti-viral activities. Ground beans lectin acts as hemagglutinating agent due to presence of polygalacturonic acid and also curative agent of Hepatoma (HepG2), Leukemia (L1210) and Leukemia (M1). Presence of all these special and beneficial properties makes them excellent drugs for the treatment of AIDS with no harmful effects as compare to synthetic drugs [17]. Beans, chickpeas, lentils, tofu and low-fat dairy products are also good sources of protein, as well as other health-enhancing nutrients like antioxidants and fibers (**Figure 2**).

2.2 Cereals

Grains and seeds, provide not only the major portion of the energy for human populations throughout the world, but also play an important role of nutritive and pharmaceutical carriers. These are filled with nutrients vitamins, proteins, minerals, fibers and essential trace elements which grow nutraceutical values and pharmaceutical applications. To increase the human health awareness, we must have to enlighten the importance of easily available seeds and grains in our food. The cereal grains like rice, wheat and corn are staple foods in several parts of the world.

Figure 2.
Legume Seeds.

Wheat is the key source of nutrition for many organisms and wheat proteins are one of the extremely used dietary proteins globally. Gliadins and glutenin are the foremost storage proteins of wheat and are deposited in distinct protein bodies in the starchy endosperm cells of the developing grain. The protein part of wheat accounts for up to 80% of the total grain nitrogen (**Figure 3**) [18].

Shewry and Tatham, in 1990 [19] reported the presence of prolamins in grains such as wheat, corn, rice and oats which are nutritious and healthy proteins to promote growth because the complete AA sequence of all major protein groups allowed for the redefine of their classification in relation to organizational and evolutionary relationships. The stored seed proteins are secreted and stored in separate protein bodies. However, the origins of the protein bodies and the mechanisms that regulate the transfer and transfer of proteins are still partially understood but the physical stability within the Golgi appears to be important, leading to the formation of electron dense clusters forming the contents of dense vesicles. The final protein Globulin contains understandable pro-domains that provide vacuolar targeting, but the inaccessible components within the sequence of mature proteins may be significant [20]. Oats are a hearty, gluten-free and inexpensive cereal.

Whitehead et al. [21] reported, much higher in protein in oats than other grains. A 1/2-cup (78-gram) serving provides 13 g of protein. Oats are high in vitamins and minerals, plus contain a type of soluble fiber called beta-glucan. Studies have shown that foods rich in beta-glucan may help reduce LDL and total cholesterol levels,

Figure 3.
Cereals.

making oatmeal a great choice for heart health. Choosing a bowl of oatmeal for breakfast is a great way to keep our heart healthy while increasing protein intake.

In maize seeds 10% proteins are there and out of these 10% nearly 70% of them are categorized as storage proteins [22]. Albumins, globulins, glutamines, and prolamins are four groups of proteins based on their solubility. The protein of maize grain varieties contains nearly two times lysine and tryptophan, AAs that are essential for humans and monogastric animals. These two AA allow the body to synthesize complete proteins, thereby eliminating wet-malnutrition, moreover Trp can be converted in the body to niacin, which is supposed to reduce the incidence of Pellagra.

Among cereals rice has lowest protein content (7%), bran layers and embryo are wealthier in non-starch constituent than the milled (white) rice. Protein from rice reduces both cholesterol and triacylglycerol levels in the liver, suppressing ability of fatty acid synthase, G6PD and MDH in liver and enhancing those of lipoprotein lipase and hepatic lipase [23]. Rice protein is also rich in Gln, Asn like other cereal proteins.

Major AAs present in Teff (*Eragrostis tef*) are Glu, Ala, Pro, Asp, Leu, Val, whereas Mat, Phe, His and Arg are essentially higher in Teff than other cereals, except rice and oats. The balance between essential AAs is similar to the whole edible portion of egg protein, except for its lower Lys content. The overall AA profile of Teff can be regarded as well balanced. Teff is different from other cereals in having higher albumins, globulins and its protein which is essentially free from gluten found in wheat so demand of Teff grain foods are increasing and become important for consumers who are allergic to gluten found in wheat [24].

Protein content of sorghum grain is quite variable, ranging from 7 to 16% with an average of 11% approximately. The key proteins are prolamin (storage proteins) as present in all other cereal grains, are called as 'Kafirins' [25]. On the basis of differences in MW, solubility, structure, AA composition and sequence, Kafirins are classified into four major species. Kafirins have low nutritional quality because they have very less quantity of essential AA, particularly lysine [26]. They are poorly digestible, especially when cooked in water, as occurs during most food preparation processes [27].

Nowadays, crops are turning into factories which don't produce food but also participating in the production of monoclonal antibodies, drugs, vaccines and enzymes. For the production of protein which are pharmaceutical active firstly we have to synthesize/isolate the genes that are responsible for pharmaceutical proteins and transformation of those genes into the desired crops then transfer of those genes into the DNA of desired crops. Different plant species, an animal (a human being) or a bacterium can be the sources of these genes or transgene which are to be transferred into the desired host. The genetically modified crops are then cultivated, harvested and pharmaceutical protein produced by crop is extracted, purified and modified prior it is given to humans or livestock [5, 6].

Cereals such as Rice (*Oryza sativa*), Wheat (*Triticum vulgare*) and Maize (*Zea mays*) are pharmaceutical crops and the major staple food consumed by half of the population globally. Rice seeds have recently gained attention as bioreactors in the production of human pharmaceuticals such as medicinal proteins or peptides. Rice seed production stages have beneficial over animal cell or microbe systems as it is more economic, scalable, safe and productive. Human pharmaceuticals based on rice seed are predictable to become inventive therapies as edible drugs. Therapeutic proteins can be divided into cellular components in rice seeds and secured to harsh areas of the stomach [28]. A high nutritive value of maize [29] bound up a valuable book chapter which explains the nutritive agents of macro and micro quantity and their

effective health benefits to protects diseases. Maize is a good source of B-complex vitamins along with antioxidants such as different types of polyphenols [30].

2.3 Vegetable and oilseeds

Oilseeds are rich source of protein and due to that these are high in demand in animal and human feed. In many of countries, the attention is on brassica crops, which comprises canola, rape and mustard. Oilseeds and their ingredients developed as resolute foods or as sources of nutraceuticals deliver benefits for consumers and diet processors. Sarwar et al. [31] reported a review article entitled "The role of oilseeds nutrition in human health: A critical review" in which the nutritional value of variable oilseeds was discussed and enlightens the nutraceutical potential of oilseeds. That article was focused on the key sorts of oilseeds, their role in human health and ailments, and high lightened the new progresses that may offer even more benefits in the future. Oilseed foods from soybean, peanut, rapeseed and flaxseed are rich in protein; when assorted with other components (cereal grains), and they deliver nutritionally balanced feedstuffs [32]. Mustard seeds and its oil have customarily been used to release muscular pain, rheumatism and arthritic pain. The mustard oil also very helpful in stimulating hair growth when applied over scalp also its ground seeds perform the role as a laxative, stimulant to gastric mucosa and surge intestinal secretion [33]. The Cucurbitaceae seeds such as *Cucumeropsis mannii*, *Cucurbita maxima*, *C. moschata*, *Lagenaria siceraria* and *Cucumis sativus* and their defatted cakes are rich in proteins hence these seeds can thus be measured as cores of proteins and oils (**Figure 4**) [34].

Oilseed rape is a very valuable crop as the seed is naturally 42% oil and the meal port after eliminating the oil is about 42% crude protein. Proteins serve a variety of functions in the human body such as acting as enzymes, antibodies, and the structural components of tissues, hormones and blood protein. The main function of dietary protein is to supply AA for the growth and maintenance of body tissue. Digestion disassembles proteins into their basic building blocks AA. Furthermore, the oil is particularly of high quality and high in monounsaturated, and should logically be a premium product. Today's certain varieties of oilseed rape have been bred to provide oil that is suitable for use in cooking and food processing. Known as vegetable oil, the oil is widely used by the food industry and is now being increasingly processed for use as bio-diesel [33].

Pumpkin seeds deliver a massive amount of nutrients in a very small package. Adding these budget-friendly seeds to your diet is a smart and healthy way to increase our protein consumption. Just 28 g of pumpkin seeds contains 7 g of

Figure 4.
Oilseeds.

protein, making them an exceptional choice for a protein-packed nosh. Along with a magnificent volume of protein, pumpkin seeds also contain antioxidants like vitamin E and phenolic acids that help reduce inflammation in the body. (www.nutrition).

Oilseed protein makes a significant influence to the human alimental protein ingestion. Furthermore, oilseeds are mostly richer in Sulphur AA than legumes, but minor in lysine except for rapeseed. The nourishing value of oilseed meals depends mainly on the oil extraction process. International dietary guidelines commend the intake for their contribution of proteins, and especially of mono and polyunsaturated fatty acids as lessens the risk of chronic diseases. Back, [35] reported that G-proteins present in the oilseeds, plays a significant role in the synthesis of Omega-3-fatty acids which supports to reduced risk of cardiovascular disease and Atherosclerosis (hardening of the arteries). There are a long list of oilseed and protein present (**Table 1**), these proteins are very beneficial to human and animal health.

2.4 Summary points

- Grain and seed proteins are easily available and economic food to obtain the valuable dietary supplement protein for every person.
- Grain protein concentration, usually stated in percent of grain dry mass.
- Proteins act as a buffer system, helps our body to maintain pH values of the blood and other body fluids.
- Proteins forms antibodies to protect our body against foreign invaders.
- The recommended protein intake for an adult is usually based on body size: 0.8 grams per kilogram of body weight.
- Variations in grain protein concentration induced by weather, water and nitrogen availability, especially during the grain-filling period.
- In oilseeds, an upsurge in oil concentration is usually associated with a decrease in protein concentration.

S.No.	Oil seed Species	Major Storage Protein
1	Canola (*Brassica* species)	Cruciferin
2	Corn (*Zea mays* L.)	Zein
3	Cottonseed (*Gossypium* species)	11S protein
4	Flax (*Linum usitatissimum* L.)	12S protein
5	Hemp (*Cannabis sativa* L.)	12S protein
6	Peanut (*Arachis hypogaea* L.)	Arachin
7	Safflower *(Carthamus tinctorius* L.)	Carmin
8	Sesame (*Sesamum indicum)*	α-globulin
9	Soybean (*Glycine max)*	Glycinin
10	Sunflower (*Helianthus annuus)*	Helianthin

Table 1.
Oil-producing crops and the major storage proteins suitable for human consumption.

- Cereal proteins are less digestible by children and adults than egg and milk protein, except for wheat endosperm.

3. Conclusion

The nutraceutical values of seeds for human health need to be needed zones of recent research. Promised research should focus on probable effects of bewildering factors that may be correlated with the use of nutritive and medicinal values of seeds. This is for above cited data suggest that seeds have valuable applications in the inhibition and treatment of numerous human diseases. Hence, it is imperative to explore not only bioavailability and chemical composition of seeds but also to characterize biological possessions to determine the mechanism as well as their synergistic assets to ensure the nutrition values for human health and make ready to overcome from pandemic and steady diseases in future.

Abbreviations

3-letter abbreviation	Amino acid
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Glu	Glutamic acid
Gln	Glutamine
Gly	Glycine
His	Histidine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
AA	Amino acid
G6PD	Glucose 6-phosphate dehydrogenase
MDH	Malate dehydrogenase

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Chapter 3

Nutritional Composition of Grain and Seed Proteins

Adeola Abiola Oso and Anofi Omotayo Ashafa

Abstract

Grains including wheat, brown rice, millet, oat, and seeds from crops such as pumpkins, almonds, cashew, peas are important staple foods in many parts of the world. Grains and seeds contain proteins and bioactive peptides classified as nutraceuticals. Proteins and peptides are essential components in man's diet because they provide the raw materials needed for protein biosynthesis and are also a good source of energy. Incorporating grains and seeds into the human diet provide nutritional, functional health benefits, reducing contracting some chronic diseases. They avail the body with a balanced nutrient profile such as carbohydrate, fatty-acid, fibre, B vitamins, minerals and protein. The quest at exploring staples for their functional and health benefits, as well as reducing risks to diseases, has resulted in the investigation of the potentials of grains and seeds, especially the underutilised ones (African yam bean, pigeon pea, Bambara groundnut etc.) for consumption and as an alternative therapy against diseases. This chapter discusses grains and seeds as sources of nutrition protagonist, their nutritive property, health benefits, and the pharmacological properties of bioactive peptides in grains and seeds. However, some under-utilised grain and seed proteins would also be explored for their nutritive potentials.

Keywords: bioactive peptides, grain, nutraceutical, protein biosynthesis, and seed

1. Introduction

Seventy-five per cent of the people in developing countries live in rural areas, especially sub-Saharan Africa and southern Asia [1, 2]. Despite intensification associated with the green revolution and expansion in agricultural production, many people remain food insecure, suffering from hidden hunger caused by protein deficiencies [3, 4]. The malnutrition problems could be addressed by exploring plant proteins as an economical and sustainable source of protein for a wholesome diet [5]. Grains and seeds are plant products containing proteins and peptides that can be classified as nutraceuticals. Nutraceuticals are any functional food extract with health and medical benefits, particularly to humans [6]. Grain and seed proteins are critical components in food systems that help combat protein-calorie malnutrition in developing countries [7]. They are referred to as the poor man's meat of the vast majority who cannot afford fish, meat and dairy since they provide nutritionally balanced protein diets [8]. Grain and seed proteins create windows of opportunities by reducing poverty level, improvement in nutrition and health status, improvement in food security and sustenance of natural resource base among

the resource-poor farming communities. Grain and seed proteins are a staple source of calories, carbohydrate, minerals, B-vitamins and proteins.

Proteins from grains and seeds are probable sources of a wide range of bioactive peptides that positively impact man's health [9]. Grain and seed high in protein include wheat, brown rice, millet, cornmeal, oatmeal, amaranth, buckwheat, couscous, teff, quinoa, whole-wheat pasta, flaxseeds, chia seeds, pumpkin seeds, peanuts, walnuts, almonds, sunflower seeds, cashews, date, kiwi, and cumin. However, the cultivation and utilization of some locally grown grain and seed proteins with potential food and nutrition security are grossly underexploited [10]. The locally underexploited grain and seed are tied to the cultural ancestry of their places of origin, acclimate to precise agroecological areas, and perform well in traditional farming systems with little or no external inputs [11, 12]. The new generation of farmers, especially in sub-Sahara Africa, have relegated the locally grown grain and seeds as crops of the older folks. Thus, the traditional farming system is exposed to genetic erosion of the germplasm of the traditional underutilized crops [13]. The formulation of production expansion strategies of the locally grown grain and seed proteins would be a step in the right direction for sustainable intensification and diversification in the global food base.

2. Grain

Grain is a member of the Poaceae family with approximately 780 genera and 12,000 species [14]. The family Poaceae is the fifth-largest plant family following the Asteraceae, Orchidaceae, Fabaceae, and Rubiaceae [15]. They possess a wide range of tolerance for climatic fluctuations; thus, they survive in almost all kinds of ecological niche [16]. The Poaceae are the most economically important plant family, providing staple foods from domesticated cereal crops [17] and feed for

Grain[1]	Protein Content (grams)	Scientific Name
Grain amaranth	6.10	*Amaranthus cruentus*
Barley, hulled	5.62	*Hordeum vulgare*
Brown rice	3.38	*Oryza sativa*
Buckwheat	5.96	*Fagopyrum esculentum*
Khorasan wheat	6.54	*Triticum turgidum turanicum*
Millet	6.96	*Pennisetum glaucum*
Oats rolled	5.92	*Avena sativa*
Quinoa	6.35	*Chenopodium quinoa*
Rye	4.65	*Secale cereale*
Sorghum	5.09	*Sorghum bicolor*
Spelt	6.56	*Triticum aestivum spelta*
Wheat	6.93	*Triticum aestivum*
Wheat, bulgur	5.53	*Triticum durum*
Wild rice	6.63	*Zizania latifolia*

[1] *All values are based on 45 g uncooked grain – Standard FDA serving size.*
Source: Oldways Whole Grain Council and Oldways Nutrition Exchange

Table 1.
The protein content of some grains.

meat-producing animals. A grain is the tiny edible fruit of the plant, usually hard on the outside harvested from grassy crops. Grains are either referred to as true cereal grains or pseudo-cereal grains. The true cereal grains are the edible seeds of specific grasses from the family of Poaceae.

Examples of true grain cereals include wheat, oat, maize, barley, rye, sorghum, and millet. The pseudo-cereal grains are not really grains but seeds from different plant species with a nutritional composition similar to the true grains. Amaranth, buckwheat, and quinoa are examples of pseudo-cereal grains. Grain foods are consumed for their higher fibre content as well as for dietary proteins. The three critical parts of grains include; the bran (outermost layer), the germ (embryo), and the endosperm [18]. The bran is made up of fibre and B vitamins; the germ contains oils, vitamins, proteins, minerals, and antioxidants; and carbohydrates and protein are found in the endosperm. Grain foods are categorized either as whole or refined grains. Whole grains have been minimally processed and still contain the bran, germ, and endosperm [19]. Whole-grain foods are higher in B vitamins and fibre. Consumption of a whole-grain diet is associated with a lower risk of several diseases [20]. Refined grains are processed grains containing only the endosperm [21]. Refined grain foods are lower in B vitamins and fibre but higher in foliate. However, vitamins and minerals (specifically iron and folic acid) lost during processing are added to the refined grain to make it healthier [22]. The protein content in most of the popular grains is shown in **Table 1**.

3. Seed

A *seed* is an embryonic plant covered in a seed coat formed from the ripened ovule of the plant after fertilization. The seed comprises three major parts - the embryo, seed coat, and the endosperm [23]. The embryo is the most crucial part because the various tissues that make up the plant are developed from its cells. The endosperm contains the nutrients while the seed coat protects the embryo. The plant seed is not only an organ of propagation and dispersal but also a significant source of dietary protein [24]. The seed contains the complete profile of amino acids needed for the formation of complete and digestible protein. The amount of protein present in seeds vary from~10% (in cereals) to~40% (in particular legumes and oilseeds) of the dry weight. Although the individual protein in seeds either play structural or metabolic roles, seed proteins generally provide a store of amino acids available during germination and seedling growth [25]. Seeds also contain vitamins A, B, C, and E and the minerals calcium, magnesium, potassium, zinc, iron, selenium and manganese. Seeds are edible, and they form the primary source of the majority of human calories when consumed as legumes, cereals and nuts [26]. *Plant seeds* are a versatile food, used to flavour a stew, as a garnish, in salads and soups. Seeds are a low gastro-intestine (GI) food and help to keep blood sugar level stable. Seeds provide many beverages and spices, cooking oils and some important food additives.

4. Nutritional properties and health benefits of some selected grain and seed

The nutritional property of food is the measure of a well-balanced ratio of the essential nutrients, carbohydrates, fat, proteins, minerals, and vitamins with the nutrient requirements of the consumer. A healthy diet supports average growth, development and ageing. It also helps to maintain a healthy body weight and

reduces the risk of chronic diseases. The nutritional properties and health benefits of some selected grains are discussed below:

4.1 Barley

Barley is a versatile grain consumed as whole grain (hulled) or pearl barley (refined). Whole grain barley contains a range of vitamins, minerals and other beneficial plant compounds. Barley is packed with fibre and lignans, a group of antioxidants linked to a lower risk of chronic Western diseases [27]. Barley is naturally cholesterol-free and low in fat [28]. It helps to reduce the risk of heart disease, prevent the development of type 2 diabetes, and aid regularity. Barley is a primary source of many nutrients, including molybdenum, manganese, dietary fibre, vitamin B1, chromium, phosphorus, copper, selenium, riboflavin, folate, iron, magnesium and niacin. Barley contains a soluble fibre known as beta-glucan, which forms a gel-like structure in the guts. Beta-glucan slows the digestion and absorption of nutrients, thereby curbing hunger and promoting fullness in man [29]. The high fibre content of barley helps to boost intestinal health [30]. The insoluble fibre in barley helps to prevent the formation of gallstones, aiding the proper functioning of the gallbladder [31]. Whole barley has a nutty flavour which makes it a great addition to soups and stews.

4.2 Sorghum

Sorghum is an old cereal grain of the family *Poaceae* considered a traditional crop of Africa and Asia [32]. It is small, round, and usually white or yellow grain favoured by farmers due to its tolerance to drought, heat, and other edaphic conditions [33]. Whole grains of sorghum contain approximately 89–90% dry matter (DM), 8.9–15% crude protein (CP), 2.8% ether extract, 1.5–1.7% ash, 2.1–2.3% crude fiber [34]. Protein, oil, niacin, and pyridoxine content of sorghum are highest in the germ fraction and lowest in the bran, while the endosperm contains the highest level of starch [35]. Sorghum is packed with a huge amount of carbohydrate, protein, fat, calcium, vitamin B1, and a small amount of nicotinic acid. It is also an excellent source of riboflavin, thiamin and minerals such as iron, potassium, manganese and magnesium. The B vitamins in sorghum play essential role in metabolism, neural development, skin, and hair health [36]. Sorghum is high in antioxidants such as flavonoids, tannins, and phenolic acids, which help to lower oxidative stress and inflammation of the body [33]. Sorghum is naturally gluten-free and a good option for people with underlining ailments such as celiac disease [37]. Sorghum syrup is widely used as a sweetener in the food industry due to its low total sugar content [38]. Sorghum is versatile, and it is available in milled flour, syrup, and whole or flaked form.

4.3 Quinoa

Quinoa is a tiny, light bead textured grain and contains all nine essential amino acids. Quinoa is gluten-free, high in protein, fibre, magnesium, B vitamins, potassium, iron, calcium, and beneficial antioxidants [39]. As an edible seed, quinoa is increasingly becoming important due to its high nutrient value and its potential to contribute to food security [40]. It is a good source of magnesium, which protects against osteoporosis. Quinoa contains many potent plant antioxidants, including flavonoids (quercetin and kaempferol) reported with anti-inflammatory, anti-cancer, anti-viral, and anti-depressant effects [41]. Quinoa is much higher in fibre

than most grains, but most of the fibre is insoluble. Substituting quinoa for other gluten-free ingredients in food recipe increases the nutrients and antioxidant value of a man's diet [42]. Quinoa is high in fibre, protein and has a low glycemic index. These properties have been linked to weight loss and healthy living [43]. Quinoa grain is roasted and processed to make different types of bread. It is prepared with strong-flavoured vegetables such as kale, spinach and red peppers. It can also be added to soups, used as a cereal, made into pasta or even fermented to beer [40].

4.4 Brown rice

Brown rice is considered as a whole grain food recommended as a healthy diet. The brown colour is from the bran, and germ layers left intact after harvesting the rice. Brown rice is highly nutritious, providing the body with an array of vitamins and minerals, including carbohydrate, fibre, fat, protein, potassium, B vitamins, magnesium, zinc, iron, selenium, and manganese [44]. Brown rice is exceptionally high in manganese, a vital mineral for body processes such as bone development, blood sugar regulation, and wound healing, amongst others [45]. The consumption of fibre-rich brown rice helps reduce belly fat and enhances weight loss [46]. The brown coat is responsible for its nuttier taste and chewy texture. It is also a good source of bioactive peptides [47]. Brown rice is naturally gluten-free and can be made into wholesome gluten-free products such as crackers and pasta.

4.5 Wheat berries

Wheat berries these are oval-shaped, chewy textured whole wheat kernel with a robust and sweet taste. Wheat berries are high in fibre, protein, iron and packed with an array of micronutrients, including manganese and selenium. Wheat berries are a good source of dietary fibres that protect against intestinal ulcers and improve irritable bowel syndrome symptoms [48]. Incorporating wheat berries into diet protects against diabetes [49]. Diets rich in whole grain like wheat berries reduce the risk of obesity [50]. Wheatberry is rich in iron and promotes healthy red blood cell production. Wheat berries enhance subtly flavoured foods, such as chicken and shellfish. Wheat is a good source of bioactive peptides [51]. When combined with other whole-grain to form a well-balanced and healthy diet, wheat berry can significantly influence many aspects of overall health. Wheatberry can be cooked and used to ad a crunch to dishes, ground into wheat flour, or grow into wheatgrass.

4.6 Buckwheat

Buckwheat is a pseudo-cereal ground into flour. There are two types of buckwheat: common buckwheat (*Fagopyrum* esculentum) and Tartary buckwheat (*Fagopyrum tartaricum*). The dietary components of buckwheat include carbohydrate, protein, fibre, various minerals and antioxidants. The fibre content of buckwheat is minimal, and it is suitable for colon health [52]. The protein in buckwheat is rich in the amino acids lysine and arginine. Buckwheat protein tested in animals has proven effective at lowering blood cholesterol, reducing the risk of colon cancer, and suppressing gallstone formation [53]. Buckwheat has higher minerals compared to other pseudo-cereals and cereals. The most abundant minerals in buckwheat include magnesium, copper, manganese, iron and phosphorus [54]. Buckwheat is rich in various antioxidant plant compounds, including rutin, quercetin, vitexin, and D-Chiro-inositol [55]. The nutty, bitter flavour of whole-grain wheat flour is delicious in chocolate chip cookies and gluten-free pastries.

4.7 Oats

Oats a vital cereal crop with high dietary fibre content and nutritive value [56]. Oat consumption is beneficial to man because it possesses quality protein with the right amino-acid balance, minerals, vitamins, dietary fibres, including functional protein, lipid, starch components ß-glucan and phytochemicals [57]. Oats are high in antioxidants, including avenanthramides. These compounds help reduce blood pressure and have anti-inflammatory and anti-itching effects [58]. The health benefits associated with the nutritional fibres have increased interest in its use as a food ingredient in various food products by the food industry [59, 60]. Food products derived from oat include oatmeal, porridge, granola bars, bread, biscuits, cookies, oat-based probiotic drink, oat-based breakfast cereals, flakes and infant food.

4.8 Grain amaranth

Grain amaranth is not a true grain but contains all nine essential amino acids missing from most grains. Amaranth is a good source of bioactive peptides [61]. Niacin, riboflavin and thiamine are essential micronutrients present in grain amaranth. These micronutrients enhance proper blood circulation, healthy functioning of the nervous system, maintenance of the gastrointestinal tract and proper metabolism of proteins and carbohydrate [62]. Grain amaranth is rich in protein, carbohydrate, fat, ash and energy needed for healthy living. It also contains essential minerals, namely zinc, iron, magnesium and manganese. These minerals stabilise the immune, alleviate anaemic conditions, and enhances the infant's growth [62]. Grain amaranth is popular in gluten-free baking as muffins and puffed granola.

5. Nutritive properties and health benefits of some selected underutilised grains and seeds

5.1 African yam bean *(Sphenostylis stenocarpa)*

African yam bean (*Sphenostylis stenocarpa)* is one of the under-utilised hardy, cheap, protein-rich legume indigenous to Africa with great medicinal values [63]. The plant, when harvested, can be consumed as seed and tuber [64]. African yam bean seed contains protein with a value range between 19 and 30%. The seed is also rich in dietary fibre, carbohydrate, and essential minerals such as calcium, iron, zinc, and magnesium, with values as high as those of other vital legumes [65]. The carbohydrate composition of African yam bean is majorly starch with slowly digestible properties beneficial for diabetic patients [66]. African yam bean is also a good source of non-starchy polysaccharides, reducing the risks posed by cardiovascular disorder, coronary heart diseases, cancers, type 2 diabetes, and other lifestyle disorders [67]. African yam bean seed has a low-fat content when compared with crude legumes such as soybean and groundnut. The low-fat content of African yam bean seed makes it ideal as a promising food crop for weight management [66]. The prevalent amino acids in African yam bean include aspartic acid, glutamic acid, leucine and lysine. The fortification of protein-deficient cereal-based diets with African yam bean addresses kwashiorkor and marasmus among infants [68]. It is a hearty food in west Africa, where millions are suffering from protein-energy malnutrition. African yam bean is used to fortify and enrich foods low in protein to address the problem of protein malnutrition [64]. African yam bean is used as composite flour with rice and brown cowpea seeds, breakfast meals, maize-African yam bean meal composite, African yam bean enriched fufu, traditional snack food, and as imitation yoghurt [64, 69].

5.2 Bambara groundnut (*Vigna subterranean)*

Bambara groundnut (*Vigna subterranean*) is the third most important in most parts of Africa legume after peanuts and cowpeas. Bambara seeds (ripe or immature) are nutrient-rich and unusually high in amino acid, with more methionine than other grain legumes. They contain approximately 64.4% carbohydrate, 23.6% protein, 6.5% oil, 5.5% fiber, and are rich in micronutrient [70, 71]. Bambara groundnut is a good source of magnesium, calcium, iron, zinc, and potassium [32]. Bambara seeds and flour are used to produce myriads of traditional foods in Africa [72]. It can be used as a condiment in cooking, making flour or eaten as a snack. Bambara groundnut can be pounded into flour and used to make a stiff porridge. Raw and cooked seeds of Bambara groundnut have an abundance of epicatechin and catechin flavonoids [73]. Catechin and epicatechin polymerize to form proanthocyanidins, also known as condensed tannins. Proanthocyanidins are documented with nutraceutical properties such as cardioprotective, antitumor, antioxidant, and neuroprotective properties [74]. The nutritional profile of Bambara groundnut sustains the growth of probiotics (live microorganisms which confer certain health benefits on their hosts). These benefits are therapeutic, suppressing the growth and activity in conditions like infectious diarrhoea, irritable bowel syndrome, and inflammatory bowel disease [75].

5.3 Pigeon pea (*Cajanus cajan)*

Pigeon pea (*Cajanus cajan*) is mainly cultivated as edible seed grain and an alternative source of protein among farmers in lean times [76]. Pigeon pea is a good source of protein, dietary fibre, and various vitamins: thiamin, magnesium, phosphorus, potassium, copper, and manganese. Pigeon pea is also low in saturated fat, cholesterol, and sodium. Pigeon pea is a good source of protein, dietary fibre, and various vitamins: thiamin, magnesium, phosphorus, potassium, copper, and manganese. The potassium found in pigeon pea is best described as a vasodilator; it helps reduce the constriction of blood vessels, thereby lowering the risk of hypertension and other cardiovascular diseases [77]. Pigeon pea has a densely packed protein content responsible for routine healing and regeneration of cells in the human body. Pigeon pea has high folate levels, which helps prevent anaemia and nueral tube defects in unborn babies [78]. Pastes from mashed pigeon pea is used in traditional medicine for the treatment of haemorrhoids [79]. Pigeon pea is low in saturated fat and cholesterol and moderate in terms of dietary fibre content.

5.4 Winged bean (*Psophocarpus tetragonolobus*)

Winged bean (*Psophocarpus tetragonolobus*) is an underutilised, nutrient-rich legume with potential as a significant multi-use food crop. Winged bean seed contains high dietary protein due to its amino-acid content, substantial protein bioavailability, and low antinutritional factors [80]. The carbohydrate content in unprocessed winged bean seed is higher than in processed winged bean seed [81]. The moderate carbohydrate content in winged bean flour makes it a good source of energy in breakfast formulations. The crude fibre content of winged bean seed is reported higher than that of most legumes. The seeds can be functional food with health benefits associated with soluble and insoluble fibre [82]. Winged bean seed can be dried and ground into flour and brewed to make a coffee-like drink. Winged bean is rich in protein and tocopherol, facilitating the utilisation of vitamin A in the body [83].

5.5 Mung bean

Mung bean is a substantive source of dietary protein containing a greater quantity of essential amino acids. Mung bean's palatable taste and high nutritional quality have endeared it as an iron-rich dietary source for infants and children. The dry weight of mung bean is composed of 20–25% protein, 55–65% carbohydrate, and vitamins and minerals. Mung bean contains much health benefiting bioactive compounds. The compounds are responsible for the antidiabetic, antihypertensive effect, anti-tumour, anti-inflammatory, and anti-mutagenic properties of the mung bean [84]. Mung bean is consumed as a fresh salad, vegetable, or ordinary food, and it is used to alleviate heat stroke [85]. The paste made out of mung bean can be used to relieve itching, treat acne, eczema and dermatitis [86].

6. Nutritive properties and health benefits of some selected seed

6.1 Flaxseeds

Flaxseeds is one of the best sources of plant-proteins and it contains omega-three fatty acids. They are also rich in vitamins and minerals such as magnesium, phosphorus and copper. Flaxseeds are rich in lignans (plant compounds with antioxidant and oestrogen properties), which lowers cancer risk and relieves menopausal symptoms. Flaxseed contains both soluble and insoluble fibres, which are worked upon by the bacteria in the large bowel, bulk up stools to allow regular bowel movements. The soluble fibres increase the intestine's consistency and slow down the rate of digestion. The insoluble fibres aid with the prevention of constipation by allowing more water to bind up the stools, increase their bulk to allow for softer stools. Flaxseed protein helps to improve the body's immunity, lowers cholesterol level, prevents tumour and has antifungal properties. Flaxseeds have health-impacting benefits such as reducing cardiovascular disease, decreased risk of cancer, anti-inflammatory activities, and laxative effects [87].

6.2 Chia seeds

Chia seeds are tiny dark seeds packed with proteins and nutrients including iron, calcium, thiamin, manganese, magnesium, zinc, phosphorus, B-vitamins, folate and riboflavin. The carbohydrate content of chia seeds is majorly in fibre, and this insoluble fibre makes humans less prone to diabetes [49]. *Chia seeds* are a high- quality plant-based protein since the seeds contain all the nine essential amino acids. Chia seeds contain beneficial plant compounds such as chlorogenic acids, caffeic acid, quercetin, and kaempferol, which help reduce chronic illnesses [88]. Chia seeds are versatile. They can be soaked and added to porridge, used in baked goods, and sprinkled on top of salads or yoghurt.

6.3 Pumpkin seeds

Pumpkins are a widely cultivated vegetable worldwide, used for human consumption and traditional medicine [89]. There are different species of pumpkins, all belonging to the genus *Cucurbita*, and are an essential source of carotenoid [90]. Pumpkin contains crispy flavourful seeds rich in amino acids. Pumpkin seed is high in protein content, iron, phosphorus and is low in carbohydrates. Pumpkin seeds are a treasured trove of vitamins, minerals and antioxidants. In traditional medicine and modern therapy, pumpkin seeds are used to treat minor disorders of the

prostate gland and urinary bladder [91, 92]. Powdered pumpkin seeds mixed with cereals are roasted, baked as bread and eaten as snacks [89]. Pumpkin seeds are rich in unsaturated fatty acids, namely palmitic acid, stearic acid, oleic acid and linoleic acid [93].

6.4 Sesame seeds

Sesame Seeds are tiny, oil-rich seeds with many potential health benefits and long-standing history in traditional folk medicine [94]. The tiny seeds, hulled or unhulled, are packed with protein, iron, zinc, magnesium, calcium and phytic acid, and low carbohydrates. Sesame seeds contain 15% saturated fat, 41% polyunsaturated fat, and 39% monounsaturated fat [95]. Studies have shown that more polyunsaturated fat and monounsaturated fat relative to saturated fat helps lower cholesterol level and reduce heart disease risk [96]. Hulled sesame seeds are a good source of protein which is a necessary building block of the body. Sesame seeds are rich in B vitamins- niacin, thiamine, and vitamin B6, essential for proper cellular function and metabolism. Sesame seeds contain sesamin – a compound with anti-inflammatory and antioxidant effects reported to soothe arthritic knee pain [97]. Ground sesame seed (sesame flour) can be used in smoothies, fish batter, baking, and more.

6.5 Sunflower seeds

Sunflower seeds are white tender-texture seeds encased in a black and white striped shell of the sunflower plant. Sunflower seeds have a distinct nutty flavour and high nutritional value – the seeds can be eaten raw, roasted or incorporated with other dishes. Sunflower seeds have a good amount of fibre, rich in protein and calories, and contain majorly polysaturated and monosaturated fats. The seeds are loaded with vitamins and minerals like sodium, potassium, phosphorus, calcium, iron, magnesium, manganese and zinc. The vitamins and minerals in sunflower seeds enhance body immunity, reduce cholesterol levels, and protect against cardiovascular diseases [98]. Sunflower seeds also contain plant compounds such as flavonoids and phenolic acids that are potent antioxidants [99]. As a natural source of zinc, sunflower seeds are immune boosters.

6.6 Almonds (*Prunus dulcis)*

Almonds (*Prunus dulcis*) are not true nuts. The edible part commonly referred to as a nut is a seed. Almonds are rich in monounsaturated healthy fats, fibre, protein and other essential nutrients. The brown layer of the almond seed contains powerful antioxidants that protect the body against oxidative-stress related diseases [100]. Almonds are high in vitamin E, which lowers the rates of heart disease, cancer and Alzheimer disease [101]. It has been documented that consumption of almonds reduces hunger and lowers overall calorie intake [102]. Almonds are used to produce milk, oil, butter, flour or paste.

7. Pharmacological properties associated with bioactive peptides in grains and seeds

Proteins and peptides derived from grains and seeds play essential roles in the metabolic functions of man and, consequently, in his general well-being. They exhibit drug-like activities and can be classified based on their mode of action as

antimicrobial, antihypertensive, immunomodulatory, and antioxidative [103]. Bioactive peptides are fast evolving as the new generation of biologically active regulators used to treat various medical conditions and increase the quality of life [104]. Pumpkin seeds contain a wide range of bioactive compounds reported with antidiabetic, antibacterial, hypocholesterolemic, antioxidant, anticancer, anti-mutagenic, immunomodulatory, antihelmintic, and anti-bladder stone potentials [105, 106]. Soybean generates bioactive peptides reported to treat induced arthritis and inflammatory bowel diseases in experimental animals [107–109]. Bioactive peptides from wheat gluten hydrolysate have been used to treat chemically-induced hepatitis in animal [110]. Rapeseed protein hydrolysate is also reported with anti-carcinogenic properties [111]. Wheat and barley exhibit the most incredible diversity and abundance of peptides with potential biological activity among the cereal proteins [112]. Also, wheat and rice have proteins with peptidic sequences showing anticancer activity. Oat derived peptides (lunasin) have been reported to have anti-inflammatory and anti-cancerous properties [113]. African yam bean is reported as a source of phytochemicals and bioactive compounds, including flavonoids and phenolic acids [114]. These bioactive compounds in African yam bean have antioxidant effects and are effective prophylactic and therapeutic compounds against several diseases. The hydrolysates of Bambara groundnut protein isolates have been reported to exhibit potent antioxidant activities and food preservative and functional food properties [115]. The bioactive peptides of Bambara groundnut isolates were also found to inhibit renin and angiotensin-converting enzyme, two components known to be associated with hypertension [116]. Peptide mixture from flaxseed with high levels of branched-chain amino acids and low levels of aromatic amino acids have been reported with antioxidant properties by scavenging 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and antihypertensive properties by inhibiting the ACE activity [117].

8. Conclusion

Grain and seed are nutritious, healthy foods that raise the nutritional effectiveness of the malnourished majority in the developing parts of the world. They are crucially important as a poor man's meat to the vast majority who cannot afford livestock products. Grain and seed contain proteins and bioactive peptides, which are referred to as active biological regulators. These proteins and bioactive peptides possess specific functional components incorporated into food products for wholesome nutrition. Besides providing healthy nutrition, grain and seed-derived proteins and peptides have bioactive ingredients endowed with protection against various degenerative diseases, promoting health and therapeutic use. Peptide-rich protein hydrolysates and bioactive peptides provide a better alternative to synthetic pharmaceuticals to prevent and treat chronic illnesses affecting many [118]. The increasing awareness of biosafety products should encourage the commercial exploration of pharmaceutic potential in naturally-derived peptides targeted at improving human health. Furthermore, peptide-rich protein hydrolysates and bioactive peptides in grain and seeds can be developed into micro and nanocapsules for inclusion in foods.

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Chapter 4

Health Benefits and Industrial Applications of Functional Cowpea Seed Proteins

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Abstract

Cowpea (*Vigna unguiculata*) is among the pulse's species of greatest economic and social importance. This legume is strategic for the food security and health of millions of people in the world. Cowpea is rich in nutraceuticals compounds such as dietary fibre, antioxidants and polyunsaturated fatty acids and polyphenols, whose health benefits and use in the food industry have been extensively studied. However, research on the identification of functional proteins from cowpea, their metabolic functions and applications in the food, health and other industries are still scarce. In this chapter, a critical review of the most recent and important research about functional cowpea proteins. We objective was identify and systematize information about the nature and functions of these proteins, as well as their use and applications in food, health and other industries. Cowpea seed proteins are highly versatile and offer direct health benefits such as reducing the incidence of cardiovascular disease and some types of cancer. The proteins of cowpea are also used in material science for the development of new technologies such as development of special fabrics for protection against ultraviolet rays and microencapsulation of ascorbic acid.

Keywords: pulse, essential amino acids, globulins, nutraceuticals, food industry, *Vigna unguiculata*

1. Introduction

The rapid increase in the cost of animal-based protein foods has increased interest in plant protein, especially from the before underutilized crops [1, 2].

The consumption of pulses (e.g., lentil, common bean, chickpea, and dry pea) generates positives impacts human and environmental health impacts, making them an ideal food for wise and conscientious global citizens [3, 4].

In fact, 2016 was declared by FAO as the International Year of Pulses, intending to heighten public awareness on the nutritional and health benefits of pulses, their biodiversity and climate changes adaptation, included in a sustainable food

production strategy designed to achieve food security and adequate nutrition [5]. In addition, pulses are a fordable and shelf-stable [4]. The American Pulse Association calls pulses the world's most versatile superfood [6].

Protein energy malnutrition (PEM) is one of the most severe public health problems in many developing countries [7]. Particularly, child malnutrition was associated with 54% of deaths in children in developing countries [8].

Cowpea (*Vigna unguiculata* L. Walp.) (2n = 2x = 22) [9] provides food for millions of people and is important in alleviating protein-calorie malnutrition [10]. Also is a good source of essential amino acids (e. g. Lys, His) and the aromatic AA [10, 11]. Because of its high crude protein content and a good balance of EAA, cowpea is usually considered as a complete food [12]. Cowpeas are also good sources of fibre, iron, zinc, and contain substantial amounts of bioactive compounds [13].

Cowpea has been promoted as a high-quality protein constituent of the daily diet among economically depressed communities in developing countries, with the aim of reducing the high prevalence of protein and energy malnutrition [14, 15]. Nutritionally, cowpea grain is the same as other pulses, with a relatively low-fat content and high total protein concentration [10].

Cowpea is a major alternative for the production of vegetable protein to be a culture of easy cultivation, low demand for soil fertility and adaptability and stability in all continents [16]. Cowpea ability to growth in low fertility and to subsist in soils where drought is a major constraint due to low and irregular rainfall confers advantages over other legume crops [17, 18].

Cowpea are also used as green manure, employed in a rotary schemewith other annual crops or in fruit plantations to increase or sustain soil fertility [19]. Dried cowpea seeds can be used for making cake or the seeds could be boiled, mixed with sauce or stew and consumed directly [20]. In addition to its great economic, social and environmental importance, cowpea is a crop with great industrial potential [21].

In the food industry, cowpea seeds is used in the production of canned and preserved foods, and in the production of isolated proteins with various applications (e.g. production or additives in flour, supplements for athletes and functional foods) [15, 19, 21–23]. However, a certain "underutilisation" of cowpea in food applications has been attributed to its beany flavour, presence of antinutrients and the hard-to-cook defect that prolongs cooking time [24].

The identification of the cowpea functional proteins and the investigation of the mode of action and application of these proteins aim to systematize information and contribute to the development of cowpea cultivation and the industrialization of this still underutilized culture, considering its great potential and studies already carried out in various areas of science.

2. Cowpea functional proteins

Vegetable proteins are presented as functional, as they provide health benefits, in addition to the essential nutrient's characteristic of the species. Functional properties of proteins are important in food processing and food product formulation. Some of these properties are water/oil binding, emulsification, foam capacity and gelation. These properties depend on characteristics of proteins such as molecular weight, amino acid composition, net charge and surface hydrophobicity [22, 25, 26].

Cowpea is a legume consumed as a high-quality plant protein source in many parts of the world [10]. It is characterized by having significant contents

of proteins (23–32%) and carbohydrates (50–60%), fibers, vitamins and nutrients, with a low-fat content (1%) and bioactive compounds, such as phenols and polyamines [27, 28].

Nutritional values and protein quality dependent on its amino acid composition, susceptibility to hydrolysis during digestion, purity and applied processing processing effects, such as heat treatments [29]. The nutritional and functional properties of pulses proteins depend on the nature of soluble fractions [12, 30]. Generally the protein content of cowpea differs along with the variety [12].

Cowpea has high protein and carbohydrate contents with a relatively fat-low content and a complementary amino acid pattern to that of cereal grains make cowpea an important nutritional food in the human diet [10]. Cowpea protein is rich in essential amino acids, particularly lysine, histidine and aromatic amino acids [31]. However, it is deficient in methionine and cysteine compared to animal proteins [32]. **Figure 1** shows the amino acid profile (essential amino acid) of cowpea protein.

The amino acid profile makes cowpea protein unique and of unquestionable quality [10]. The functional attributes of proteins like gelation, foaming, emulsification, thickening also drive the incorporation of isolated proteins in various foods like mayonnaise, baked foods and beverages [33]. The manner of converting the isolated proteins into powders also determines their functional properties [34].

In cowpea, protein types comprise globulins, albumins, glutelins and prolamins [12, 35]. Albumins and globulins are considered to represent the major storage proteins in cowpea [36]. Globulins represent most cowpea seed proteins and constitute over 51% of the total seed protein, while albumins approximately constitute 45% [37].

Glutelins have poor lysine content in cowpea [12]. Albumins has functional role in seeds as enzymatic and metabolic proteins (i.e. lipoxygenase, protease inhibitors and lectins) [38, 39]. Globulins has an important role as storage proteins and were mostly digested by proteases [38–40]. Prolamins are storage protein found mainly in seeds with high proline and glutamine content [41].

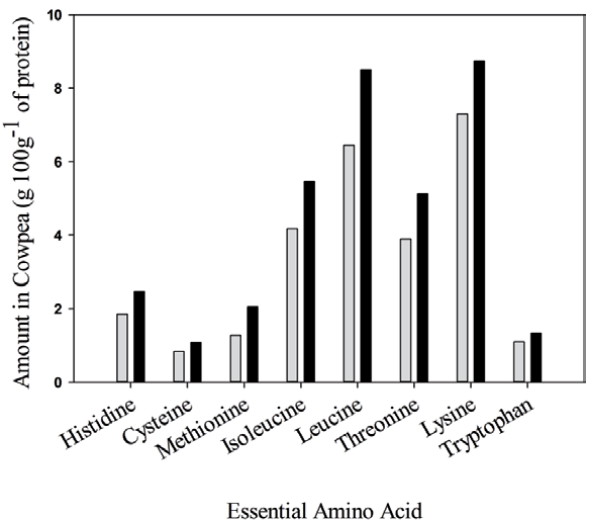

Figure 1.
Essential amino acids profile of cowpea seeds. Black bars = upper values and gray columns = lower reference values found in the literature. Adapted from [10].

3. Benefits of functional proteins of cowpea seeds for health

Vegetable-based food systems are more sustainable than meat-based ones because they require less energy, land, and water resources [10, 19, 21, 26, 42]. Proteins from pulses has advantages in terms of sustainable development, nutritional properties, and health benefits [43, 44].

Cowpea is considered as an incredible source of many other health-promoting components, such as soluble and insoluble dietary fiber, phenolic compounds, minerals, and many other functional compounds, including B group vitamin, tocopherols (i.e. E group vitamin), anthocyanins and carotenoids [45–48].

Functional ingredients in cowpea that aid in weight loss [49], improve digestion and strengthen blood circulation also reports in the literature [50]. The low glycemic index of cowpea is attributed to the action of resistant starch and dietary fiber which attenuate insulin responses and reduce hunger [51].

Consumption of cowpea exerts protective effects against several chronic diseases [52], such as gastrointestinal disorders [50], cardiovascular diseases, hypercholesterolemia and obesity [53]. Cowpea has medicine properties, including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory and anti-hypertensive properties [10, 42].

The therapeutic (or health) benefit of cowpea is principally attributed to its high protein, carbohydrate content as well as essential amino acids [54]. Cowpea proteins serve as an important ingredient in developing foods for all segments of population, however the functionality of proteins also assists in texture designing of foods [33]. Furthermore, consumption of cowpea and other grain legumes protein has been linked to reduce plasma low density lipoprotein, as well as incidences of cardiovascular diseases and some types of cancer [22, 55, 56].

4. Industrial use of cowpea seeds functional proteins

The cowpea is an annual pulses with high content of dietary protein rich in essential amino acids such as leucine, lysine, phenylalanine, tyrosine, aspartate, glutamate and arginine [11]. Their value as ingredients in food products is determined by their functional properties and nutritional characteristics [19].

Cowpea seeds utilization has been mainly limited to traditional uses [57]. Nevertheless, cowpea has the potential to become an industrial crop and the widespread consumption of convenience foods containing significant amounts of cowpea has substantially increased the demand for cowpea grain [22, 58, 59]. Due to the techno-functionality of its proteins, cowpea acts as an interesting ingredient [60, 61] for the food industry and others.

During the processing of cowpea seeds to produce ingredients (e. g. flour, isolated protein), there may be a breakdown or denaturation of legume proteins due to treatment conditions, including high temperatures, pH and osmotic potential [19].

These functional properties in cowpea seeds are influenced by environmental variables (e.g. temperature), pH and ionic strength during protein isolation and, also, during food processing, manufacturing, storage and preparation [31, 62].

Several methods of processing cowpea being studied, including treatment with temperature or high hydrostatic pressure in cowpea protein isolates. The modified cowpea protein isolates can be used in beverages because of the high solubility, in desserts because of the gel-forming ability and / or as additives in other foods because of the improved water holding capacity [19, 42].

Cowpea protein isolates (CPIs) can be used as ingredients and supplements [19]. It is not by chance that the food industry is the one that most industrializes

Protein	Action/aplication	Reference
7S and 11S globulins	Antibacterial agents	[66]
	Meat preservative	[13]
	Texture improvements in comminuted fish and meat products	
Cowpea isolates proteins (CPI's)	Applications for enhancing wettability and UV-Protection properties	[67]
CPI's	Antioxidants and aid in cancer prevention	[11]
	High potential as candidates for the therapeutic intrusion of cancer	[54]
CPI's	Microencapsulation of ascorbic acid (AA)	[51]
CPI's	Antifungal activity with application in bread	[68]

Table 1.
Identification and industrial application of cowpea proteins.

cowpea. In addition to high protein content, cowpea has proteins and functional peptides with different properties (e.g. gelafication and emulsification), molecular and non-molecular antioxidants, such as tochochromanols (i.e. different forms of vitamin E [63]), also important for the preservation of food and stabilization of several beneficial substances during the handling and packaging of processed food products produced with cowpea seeds.

Cowpea seed consumption is limited by their low digestibility, deficiency of sulphur containing amino acids and presence of antinutritional factors such as trypsin inhibitors, oligosaccharides (e.g. raffinose, trealose, staquiose) and phenolic compounds [10]. Adequate processing methods can be used to destroy those antinutritional factors, and improve the bioavailability levels [15].

A simple and inexpensive way to modify protein structure is to increase the pH of protein extraction during protein isolation. This treatment increases protein yield and influences chemical profiles of other compounds present in protein isolates [17, 64, 65]. In addition to the food industry, other industrial sectors have used and benefited from cowpea drinking proteins (**Table 1**).

5. Conclusions

Functional cowpea proteins are widely used in the food industry, which concentrates the largest number of researches. However, the use of these proteins in other industrial sectors, such as the medical and materials industry, which is still little explored, is beginning to grow. Future research should focus on the development and application of inputs and products for these industries.

Conflict of interest

The authors declare that there is no conflict of interest in the production and publication of this chapter.

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Chapter 5

Advances in Food Development with Plant-Based Proteins from Seed Sources

Isaac O. Daniel and Mulualem T. Kassa

Abstract

Increased awareness on the effects of food on human health and the environment has compelled the need to look for alternative food sources. This resulted in the steady increase in demand for plant-based protein foods as opposed to animal food sources on the premises of significant health benefits, environment-friendly sustainable production systems and moral ethics. This trend has also been reflected in recently reviewed national food guides. Research on plant-based food systems primarily aims to understand the nutritional and functional roles of dietary proteins sourced from crop seeds. Recent scientific advances in this field explore the use innovative technologies in the research and commercial applications of seed proteins. The objective of this paper is to review and summarize key research efforts and recent advances on the utility of seed-sourced proteins in the food product development applications. Important topics covered in the review are: exploration of sources of dietary protein seeds, the status of seed dietary protein research for nutrition and health, and the deployment of new and innovative technologies for developing dietary seed proteins. The topics draw on research and publications on the availability, functionality, quality, genetics, and innovative technologies to develop value-added products from dietary plant-based proteins. The review will fill knowledge gaps in the utilization of emerging plant-based protein food systems in relation to nutritional and health benefits, process technologies and promoting food system sustainability.

Keywords: dietary proteins, grain sources, essential amino acids, protein bio-availability, bioactive peptides, protein functionality, plant protein genetics

1. Introduction

Proteins are in the class of biological macromolecules which are necessary for virtually all activities in living organisms as they engage in complex interactions among themselves and other macromolecules like polysaccharides and nucleic acids to drive cellular functions. In this sense, protein intake from food sources plays essential biological roles in the diets of humans and livestock. Among the three macronutrients (carbohydrates, fats, and proteins), protein insufficiency and deficiency in diets has been found to cause more anomalies to human health and wellbeing [1, 2]. Food-derived health issues constitutes the new threat to global food security and human health. The Food and Agriculture Organization (FAO) of

the United Nations estimated that about 15% of the world's population is chronically hungry due to nutritional inadequacy [3]. Gosh et al. [4] estimated that about 1 billion people face nutritional insecurity, suffering from myriads of nutrient deficiencies and poor health because of insufficient protein intake.

Until recently, dietary proteins have been sourced primarily from animal products including meat, eggs, dairy, and blood. However, the production of dietary proteins from animal food sources is raising adverse ecological footprint concerns. In addition, there is a need to double the present global food production by 2050 [5]. Meeting this challenge in environmentally sustainable ways compel the search for alternative protein sources. The body of literature that quantifies sustainability of animal-based versus plant-based agroecosystem models is growing and most of them found better sustainability in plant-based protein food system [6]. For example, Eshel et al. [7] estimated that by replacing meat proteins with plant alternatives, the US could save 35–50% of the Greenhouse Gas (GHG) emission. Besides this, the cultural practices in animal protein production systems are known to depleting non-renewable resources like phosphorous. Continuing the current rate of phosphorous consumption required in animal production operations was estimated to potentially depleting the limited reserves of the world's phosphorus within 50–100 years [8, 9]. Hence, besides health challenges, findings in the environment frontier warrants further research on plant-based protein alternatives.

The plant-based dietary protein supply is being sustained by the grain commodity markets. Grains constitute important ingredients of the diets of livestock and humans. Generally, grains are botanically the seeds of cereals, pseudo-cereals, and legumes commodity crops [10]. Most of the commercially available plant protein foods in the industry are made from ingredients containing crops of each of these classes of grains. A visualized analysis of FAO's [11] food production datasets in the last decade showed steady growth in the value of food ingredients used in the plant-based protein industry using the pseudo-cereals, legume and cereal crops groups (**Figure 1**). This data suggests that the availability of grain commodities in commercial quantities enable the market to meet the raw material demand for production of plant protein products.

The dominance of cereal crop production value does not necessarily interpret to growth over the years. The steady growth in the value of legumes over the last decade indicates value addition of these crops due to the shifts in the

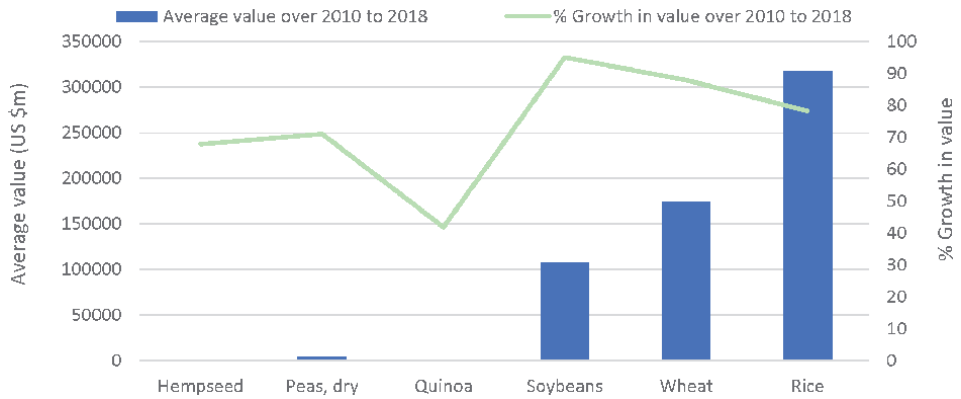

Figure 1.
Value of major grain commodities used as ingredients for producing plant-based protein foods over the last decade. Data adapted from FAOSTAT [11].

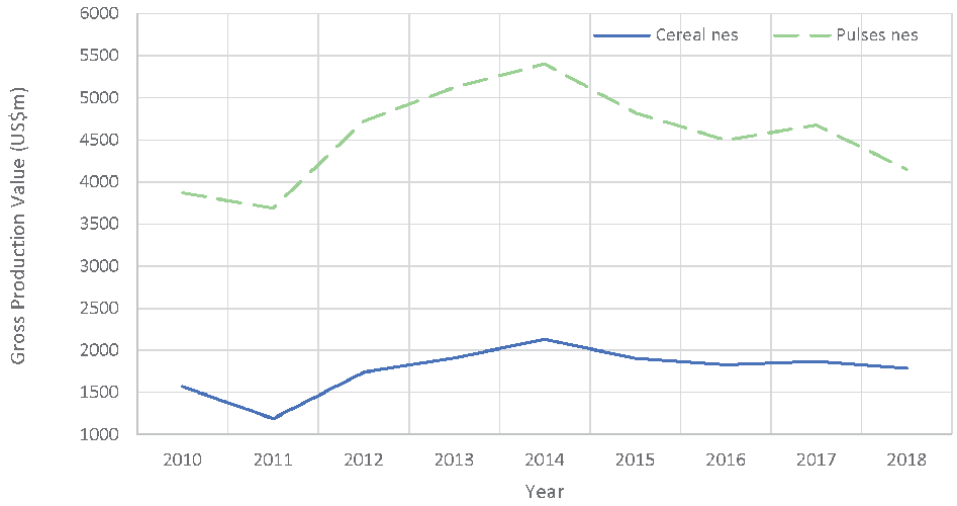

Figure 2.
Gross production value of cereals and pulses grain crops over the last decade. Data from FAOSTAT [11].

consumption of plant dietary protein sources. Over the years, growing concerns over the health implications of gluten diets common in wheat and other cereal crops compels the need to diversify the sources of plant-based proteins. For example, an analysis of the grains production dataset of cereals against pulses over the decade shows that global cereals production trails behind that of leguminous pulses (**Figure 2**), depicting the shift to gluten-free diets and the revolution of consumption of high protein crops. The consumption pattern also depicts the research investment in diversifying the sources of plant-based foods with protein composition that are suitable for the production of gluten-free foods. Moreover, concerted research efforts tend to focus on enhanced health benefits [12, 13]. Along these trends comes the growing knowledge in grain processing for plant-based protein diets, with ripple effects on research-intensive regulatory policies [14, 15].

The aim of this chapter was to review recent studies on food development based on dietary protein from grain sources. The review seeks to consolidate the state of knowledge in the actively growing field of plant-based proteins that has elicited numerous publications, innovations and technologies in the last few years. In this review, we probed PubMed and associated libraries along with other sources of compelling information or datasets like FAO and WHO etc. The keywords for the calls in PubMed contained "plant-based seed proteins", covering 2010 to 2020. We probed four research themes - crop source exploration and diversification, health and functional food development, product improvement through processing for functionality, and crop genetics (**Table 4**).

2. Exploration of dietary protein sources

In this section, we shall explore the scope of crop exploitation for the production of seed dietary proteins *vis-a-vis* the development of value-added products in the food industry. It should be noted that while the authors of this chapter recognize the broad diversity of seed protein sources in the plant kingdom, the main focus of this chapter is plant protein sources from the grains, which invariably constitutes the dominant input of the plant-based protein food industry.

2.1 Comparative sources of dietary proteins

Recently, the evaluation of protein quality shifted from raw weight or caloric estimates of food dietary content to estimates of nutrient value in foods. The emphasis of dietary protein quality now tends to be based on the bioavailability of individual nutrients measured in terms of true digestibility of amino acids, namely, the essential amino acids (EAA) content retained after digestion [16, 17]. EAAs are the amino acids that humans and experimental animal models do not produce in sufficient amounts *de-novo*, and so they must be acquired from food sources. There are nine EAAs namely; leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine and histidine. Fürst et al. [18] introduced the concept of conditionally indispensable amino acids in terms of adequacy especially in relation to disease conditions, thus extending the list of EAAs to include arginine, cysteine, glutamine, proline, and tyrosine.

Many studies that evaluated animal or vegetal foods for dietary proteins established that plant-based proteins have unbalanced EAA nutritional value when compared with animal-based sources [18, 19]. Growing evidences from research are however showing that the EAA content of some seed-sourced proteins are quite comparable to those of animal sources. **Table 1** shows data from a recent review of studies that compared amino acid profiles of selected high-protein seeds from cereals (wheat), legumes (soybeans), and a pseudo-cereal (quinoa) with animal food products like whey protein, casein, diary, and beef [19]. The EAA content is considerably comparable between both food sources. Though the findings have generated ambiguity in comparing protein dietary sources, some answers to this puzzle are coming from the accuracy of measurements of protein food quality in terms of the metrics of digestibility and bio-availability of their EAAs.

The measurement of protein quality in terms of digestibility and bioavailability of EAAs was revised in the early 1990s to 2012 from Protein Digestibility Corrected Amino Acid Score (PDCAAS) to Digestible Indispensable Amino Acid Score (DIAAS) [21, 22]. PDCAAS was dropped because of concerns in the capacity to

	Plant-Based Proteins			Animal-Based Proteins			
	Wheat	**Soybeans**	**Quinoa**	**Whey**	**Casein**	**Milk**	**Beef**
	Essential amino acid scores (% total protein)*						
Histidine	2.1	2.6	3.1	1.9	2.7	2.7	3.6
Isoleucine	4.1	4.7	4.7	6.4	5.0	5.1	5.0
Leucine	6.8	8.0	7.8	9.9	8.9	9.5	8.5
Lysine	1.4	6.6	7.2	9.2	7.6	6.9	9.3
Methionine + Cysteine	1.6	1.3	2.6	2.0	2.6	2.5	2.8
Phenylalanine + Tyrosine	5.1	5.1	5.3	3.8	4.9	4.6	4.4
Threonine	2.5	4.0	4.5	6.7	4.3	4.0	4.8
Valine	4.2	4.9	6.1	6.3	6.3	6.2	5.2

** Scores were calculated based on EAA recommendations for a healthy human adult [20].*

Table 1.
Essential amino acid scores (EAA) of selected animal-and plant-based protein sources. (data adapted from Gorissen and Witard [19].

accurately evaluate protein content in terms of digestibility. Firstly, PDCAAS truncates the scores at 1.00, missing out on proteins with higher digestibility values than 1.00. Secondly, its values likely overestimate protein quality since the method uses fecal analysis to obtain protein digestibility. It misses data on nitrogen disappearance in the large intestine, which is not as a result of protein digestion and absorption, but rather to microbial degradation. On the other hand, DIAAS is considered a superior measure of protein quality because it is calculated using ileal digestibility, and the values are not truncated at 1.0 [23].

DIAAS is an active area of research in the study of grain-based dietary proteins [24, 25]. However, evidences from previous studies that compare grain-based dietary proteins to animal proteins typically indicate that animal proteins have higher digestibility scores compared to plant proteins in the human gut [26–29]. One of the studies on plant-based dietary proteins compared digestibility values for four animal proteins and four plant proteins in pig guts instead of rats [29]. The researchers found that the DIAAS of most of the indispensable amino acids from animal sources like whey protein isolates, whey protein concentrate, and milk protein concentrate were significantly greater ($P < 0{\cdot}05$) than for pea protein concentrate, soya protein isolate, soya flour and wheat. DIAAS evaluation open new research vistas on the true quality of seed proteins.

2.2 Seed sources of dietary proteins

Figure 3 summarizes the amino-acid content of plant food sources of proteins as compiled by FAO. The visualized summary indicates a linear increase in protein and EAA contents from cereal sources to pulses and oilseed crops. The shift to pulses for grain-based proteins was recognized by the 68th United Nations (UN) General Assembly's declaration of 2016 as the "International Year of Pulses" (IYP) [30]. The UN-FAO in their implementation of the declaration recognized 12 types of pulses: dry beans, dry broad beans, dry peas, chickpeas, cowpeas, pigeon peas, lentils,

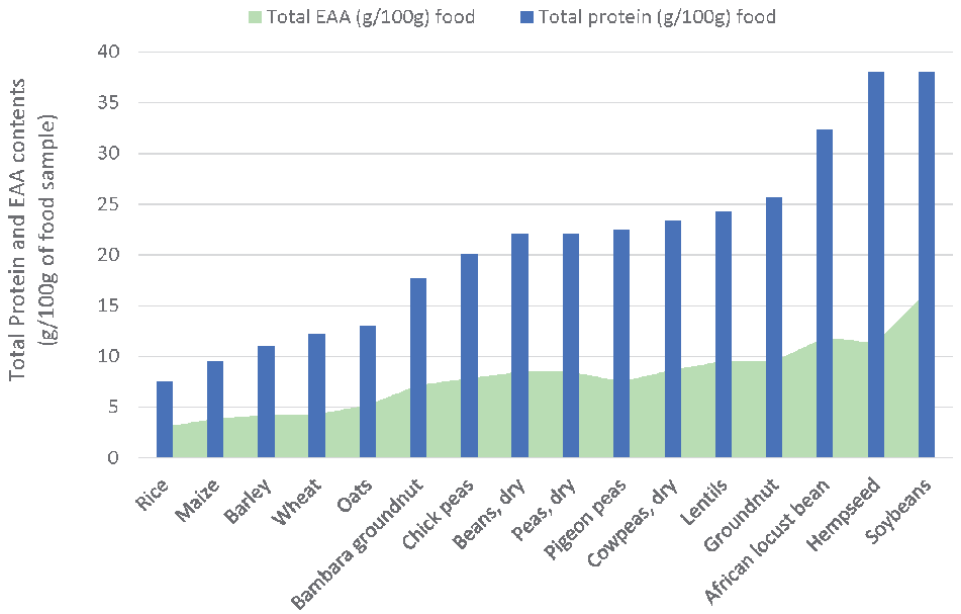

Figure 3.
Dietary protein and equivalent essential amino acids (EAA) of cereals and legume sources. Data from FAO [11].

Bambara beans, vetches, lupins and pulses nes (not elsewhere specified – minor pulses that do not fall into one of the other categories) [30]. It's known that pulses and oilseed crops like soybeans are leguminous species, which are capable of fixing atmospheric nitrogen in symbiosis with Rhizobium (nitrogen fixing bacteria). The profile of legume proteins is mainly albumin, globulin, prolamins, and glutelin in varying compositions [31]. In grain pulses, legumin and vicilins a predominant and in soybeans there are mainly glycinin and beta-conglycinin, and 2S albumin, all of which generally belongs to the globulin family of seed storage proteins [31].

Data on digestibility and bioavailability of legume proteins in terms of DIAAS is still growing. Much of what is known thus far about DIAAS scores of digestibility of EAAs from plant-based proteins comes from comparison of food proteins in the animal guts [26–29]. There are however a number of studies reported on DIAAS of legume grains in the guts of different ages of experimental animals and humans. A recent article reported a study on the true digestibility values (percentage of the total indispensable AA from ileal extracts) of some Chinese pulses. The results of the experiment in humans older than 3 years to adults shows that DIAAS was 88% for kidney bean, 86% for mung bean, 76% for chickpeas, 68% for peas, 64% for adzuki bean and 60% for broad beans [32]. In another study, Kashyap et al. [33] used the isotopic method to estimate DIAAS for mung bean and reported that the true mean ileal IAA digestibility of mung bean was 70.9 ± 2.1% after dehulling, demonstrating inconsistencies in methodologies of amino acid digestibility and indicating research gaps and need for elaborate datasets for seed dietary protein measurements to meet the quality challenge in the development of grain-based proteins [33].

As knowledge is advancing on protein quality evaluation of plant-based food sources, Herreman et al. [34] recently published a comprehensive review of DIAAS scores for 17 various sources of dietary proteins including some seed sources. The data shows that animal sources of dietary protein have high digestibility of lysine and methionine, comparable only with pea and soybeans, while the cereal sources showed the lowest DIAASS for these EAAs (**Figure 4**). The higher digestibility estimates of lysine and methionine in potatoes and hemp than cereal

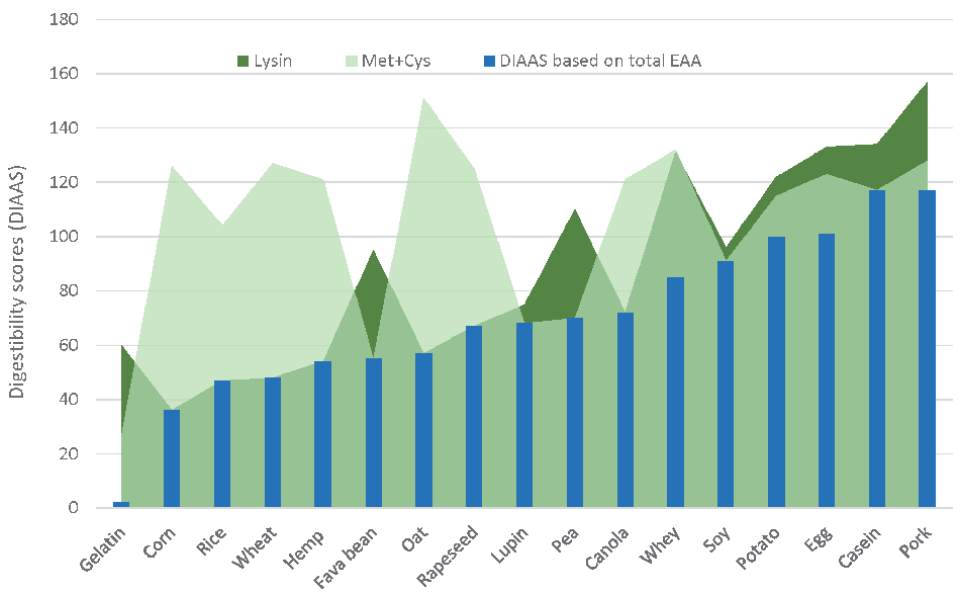

Figure 4.
Digestibility scores (DIAAS) of limiting EAAs (lysine and methionine+cysteine) and DIAAS of 17 dietary protein sources according to the 0.5-to 3-year-old reference pattern score. Data from Herreman et al. [34].

seeds and some pulses as shown in in Herreman's dataset, indicates that there are non-conventional sources of plant dietary proteins besides cereals and grain legumes. There are reports on pseudo-cereals (Amaranth, quinoa, hemp, and chia) as sources of plant-based protein ingredients comparable with animal proteins in human diet because of the special functional properties [35–37]. Other workers reported the presence of high levels of limiting EAAs *i.e.* lysine and sulfur containing amino acids (methionine + cystine) in cereal and legume proteins respectively [38–40]. Mattila et al. [41] published the nutritional values for seven plant-based dietary protein sources namely: buckwheat, fava bean, flaxseed, hemp seed, lupin, quinoa, and rapeseed. The sheer volume of plant species waiting to be explored as dietary protein sources provide opportunities for more research and reviews, especially on DIAAS, to consolidate the knowledge for scaling these research outcomes.

3. Advances in seed protein development for nutritional and health benefits

Much of the interest in plant-based protein sources are driven by health reasons. Since dietary protein and it's EAAs provide nitrogen (N), which is required to support basic metabolic processes such as protein synthesis and all other cellular activities, it's crucial to the health of the living systems. Hence advances in this area of research had been very steady in the last decade. We have reviewed a number of reports on health benefits of various grain-based proteins firstly as nutrient sources and secondly as revolutionary bio-refinery health products.

3.1 Functional foods and nutritional benefits from seed dietary proteins

Health Canada defines functional foods as "ordinary food that has components or ingredients added to give it a specific medical or physiological benefit, other than a purely nutritional effect" [42].

Because plant-based dietary proteins are not known to provide all the EAAs, Krajcovicova-Kudlackova [43] identified the risk of lower protein synthesis for vegans due to reduced lysine and indispensable Sulfur EAAs in many single plant-based proteins diets. That is same risk of falling short of the recommended daily allowance (RDA) for to achieve N-balance (i.e., N-loss = N-intake), which is about the efficient use of dietary proteins depending on Metabolic Demand (MD) [44–46]. This coupled with lower bio-availability of plant-based proteins compared to animal proteins compels the need to augment plant protein foods for limiting EAAs. This is the background for research on producing functional foods with plant-based proteins.

Recent reviews show that research in this area can be rounded up in two main strategies – protein complementation and fortification [47, 48]. It's however noteworthy that both research strategies work with protein/EAA quality evaluation in most of the projects. Protein complementation strategies have been studied in various combinations of blending foods that are deficient in certain EAAs with other ingredients that provides the limiting EAAs. Protein blending strategies can either be plant with plant sources, or plant sources with other protein sources to complement limiting EAAs. Márquez-Mota [49] found that blending low lysine cereal proteins (corn) with low Sulfur amino acids of legume (soybeans) proteins elicited improved metabolism (mTORC1-signaling pathway and hepatic polyribosome profile). Another published research strategy of plant protein complementation involves blending with protein of animals (casein, whey and diary) with plant-sourced ones (soybeans isolates or concentrates) [50–52].

Protein	Process	Bioactive peptide	Health benefits	References
Soy (β-conglycinin)	protease	Leu-Leu-Pro-His-His	Anti-oxidant properties	Chen et al. [61]
Soy	Alcalase	Trp-Gly-Ala-Pro-Ser-Leu-Leu-Pro-Tyr-Pro	Hypo-cholesterolemic activity	Zhong et al. [62]
Fermented soybean	Enzymatic hydrolysis	Leu-Val-Gln-Gly-Ser	Anti-hypertensive activity	Rho et al. [63]
Wheat germ	Bacillus licheniformis alkaline protease	Ile-Val-Tyr	Anti-oxidant properties	Matsui et al. [64]
Wheat (gliadin)	Acid protease	Leu-Ala-Pro	Anti-hypertensive activity	Motoi and Kodama [59]
Rice	Alcalase	Thr-Gln-Val-Tyr	Anti-oxidant properties	Li et al. [65]
Corn gluten meal	Alkaline protease and Flavourzyme	Leu-Pro-Phe-Leu-Leu-Pro-Phe- Phe-Leu-Pro-Phe	Anti-oxidant properties	Zhuang et al. [66]
Hemp seed protein	Pepsin	Trp-Val-Tyr-Tyr-Pro-Ser-Leu-Pro-Ala	Anti-oxidant properties	Girgih et al. [67]
Rapeseed protein	Alcalase	Leu-Tyr and Arg-Ala-Leu- Pro	Anti-hypertensive activity	He et al. [68]
Sorghum	Alcalase	Leu-Asp-Ser-Cys-Lys-Asp-Tyr-Val-Met-Glu	Anti-hypertensive activity	Agrawal et al. [69]
Wheat germ protein	Alcalase	Gly-Asn-Pro-Ile-Pro-Arg-Glu-Pro-Gly-Gln-Val-Pro-Ala-Tyr	Anti-oxidant activity	Karami et al. [70]
Pea seed meal	Gastro-intestinal digestion	Met-Val-Asp-Thr-Glu-Met-Pro-Phe-Trp-Pro	Anti-Obese activity	Ruiz et al. [71]

Table 2.
Seed protein derived bioactive peptides with antioxidant activity. Data adapted from Karami & Akbari-Adergani [60].

Berrazaga et al. [49] detailed 16 clinical studies over the last ten years that assesses nutritional and anabolic properties of plant-based protein sources in animal models and humans with various MDs involving muscle synthesis. Engelen et al. [53] reported that fortifying soy proteins with branched-chain amino acids (leucine, isoleucine, and valine) relieved muscle wasting in elderly patients with chronic obstructive pulmonary diseases elderly patients. One research question raised in this area of studies is understanding specific nutritional requirements at individual level in different stages and lifestyles. Hopefully, advances in the field of nutrigenomics will open opportunities to fill this wide knowledge gap.

3.2 Bioactive peptides and nutraceutical activities of seed proteins

Nutraceutical products are isolated or purified from foods and generally sold in medicinal forms or as a pharmaceutical alternative which claims physiological

benefits or provide protection against chronic disease [54]. Bioactive peptides have nutraceutical activities, in the intestine, they get absorbed into the blood circulation and exert systemic physiological effects in target tissues. They are sequences between 2 and 20 amino acids that have been reported to inhibit chronic diseases by playing various roles such as antioxidative, immunomodulatory, antihypertensive, hypo-cholesterolemic, anti-obesity and antimicrobial [55]. They are inactive when they are part of the parent protein sequence, but become activated upon release by *in vivo* digestion, *in vitro* enzymatic hydrolysis/fermentation, and food processing with acid, alkali, or heat [56].

Plant-based proteins are rich sources of bioactive peptides that have specific physiological and biochemical functions. Literature on bioactive peptides sources from seed proteins with physiological effects and health benefits are enormous [57, 58]. Soybeans has been the most exploited seed source of bioactive peptides with nutraceutical activities on more than 40 health conditions as demonstrated by publication on over 100 products [59]. The field of research into bioactive peptides is very active and the literature resources is vast and diverse, but we have summarized a few common bioactive peptides made from seed proteins in **Table 2** according to Karami & Akbari-Adergani [60].

4. Advances in the improvement of seeds for plant-based proteins

Researchers employ different methodologies drawn from different scientific fields towards improving plant-based proteins. The strategies for improving plant-based proteins in literature can be viewed as focused on functional improvement on the front-end and on the back-end is genetic improvement of seed protein quality traits in source crops. Investigations on the two strategies draw on mixtures of scientific methodologies. Most studies on functional improvement investigates physico-chemical and sensory properties of food products made with plant-based protein ingredients [72], while back-end studies leverage basic crop improvement methodologies that integrate various -omics techniques together with modern plant genetics and breeding. In this section, we will review studies related to the functionality of plant-based protein food products and the genetic improvement strategies of their source crops.

4.1 Seed protein analogs of animal protein foods

Plant protein analogs of animal protein foods are the most popular products in the contemporary plant-based protein gaining markets globally. Analogs are substitutes either used as whole foods of ingredients in producing either meat or dairy alternatives. Meat alternatives strives to resemble meat in appearance, texture and taste when hydrated and cooked [73], necessitating functionality and sensory research on them. Owusu-Apenten [74] defined protein functionality in foods as measuring the structure of dietary proteins in the context of their performance in food compositions. Functionality testing for food formulations differ between food types, so that the testing required for meat analogs are different from dairy analogs. While the functionality evaluation for meat products includes rheological properties, chewiness, and sensory values like color and taste [75], the functional evaluation of dairy analog products is by emulsification, foaming, gelation [76] besides sensory properties like whiteness and flow.

A review of most of the meat alternative products in the market shows that they are made from plant proteins from wheat, rye, barley, and oats containing gluten (gliadins and glutelin), soybeans containing β-conglycinin protein bodies, legumes

(prominently peas) containing glycinin and vicilin proteins; and legumin, oilseeds like Canola containing albumins, globulins, glutelin [76]. Studies on functional properties of plant proteins of meat analog products are very dynamic because the formulations differ in structural forms such as flour, protein concentrates, protein isolates, and peptides. These structural forms interact with protein contents of the ingredients, hence, research and testing of functional properties in terms of physico-chemical composition and sensory evaluation continues to be an area of active research for meat alternatives [77]. Excellent and current reviews (up to 2020) provide details of physico-chemical studies of meat alternatives in the market [76, 78, 79]. An active area of research is the investigation of reconstruction techniques of plant protein sources. Shia and Xiong [80] summarized studies in physico-chemical interactions and the aggregation of plant proteins into particles and anisotropic fibrils to impart meat-like texture; they concluded that thermo-extrusion is the principal re-constructuring technique for meat-like fiber synthesis from plant proteins [79]. Moreover, some workers are investigating digestibility as regulatory interests seeks more transparency including information on protein bio-availability in commercial meat analog products [39, 78, 81]. Kumar et al. [75] published an up-to-date review of health implications of proteins in existing meat analog products.

Diary analogs in the market are mostly milk, cheese and yoghurt products [79, 82]. There are current comprehensive reviews of functionality and sensory evaluation of diary products including milk-like foods from crop plant sources. McClements [83] compared plant-based milks with cow's milk with fortified plant -based milks. In the review, two methods of formulating plant-based milk from various crop sources; mechanically breaking down certain plant materials to produce a dispersion of oil bodies and other colloidal matter in water, or by forming oil-in-water emulsions by homogenizing plant-based oils and emulsifiers with water. The review highlighted the physico-chemical properties (viscosity and flow index), structural properties (mean particle diameter and separation rate), and sensory evaluations (whiteness) of various formulations of plant-based milks (**Table 3**). The data presented shows that the plant milk analog composition have comparable values in structure, optical properties, rheology, stability, and digestibility with cow's milk (**Table 3**). Martinez-Padilla

Milk	Viscosity [mPa•s]	Flow Index	$^{*}D_{3,2}$ [μm]	D_{43} [μm]	Separation Rate (%h)	Whiteness Index
Hemp	25.0	0.73	1.1	1.5	4.4	68.5
Oat	6.8	0.89	1.7	3.8	40.1	60.2
Quinoa	13.2	0.76	1.1	81.5	32.0	71.4
Rice	2.8	0.97	0.88	10.5	42.8	66.5
Brown Rice	2.2	1.00	0.63	0.72	50.9	63.5
Soy	7.6	0.90	0.94	1.3	11.3	70.3
Soy	3.5	1.00	0.80	1.0	8.6	74.5
Soy	2.6	1.00	0.85	1.0	13.3	69.3
Soy	6.0	0.92	0.94	1.2	22.6	74.6
Cow's	3.2	1.00	0.36	0.60	3.9	81.9

**Mean particle diameter (D_{32} and D_{43}).*

Table 3.
Physico-chemical properties of milk analogs from plant-based food sources. Data table reproduced from McClements [83].

et al. [79] also reviewed various crop sources of plant-based milk analogs for protein digestibility and compared them with cow's milk. Sim et al. [82] reviewed plant-based yogurts made by the fermentation of grain-based milks, imparting fermented flavors and probiotic cultures and thereby reducing the protein content of yogurts. The researchers addressed these challenges by exploring high-pressure processing (HPP) of plant protein ingredients as an alternative structuring strategy for the improvement of plant-based yogurts.

Though research in functional properties of these food classes continues, each emerging formulation of analogs raises research questions on functionality and quality in terms of digestibility.

4.2 Leveraging on modern genetics and breeding for seed protein improvement

The genetic improvement of seed proteins began with discovery of corn endosperm carrying the *Opaque-2* gene in homozygous recessive state [84]. This constituted the genetic background for the development of quality protein maize (QPM) parental populations with increased levels of amino acids lysin and tryptophan. Corn varieties with *Opaque-2* double recessive mutant gene are noted for up to 94% lysin content with about 90% bio-availability against 62% lysin content in *Opaque-2* heterozygous recessive corn populations [85]. For example, the Provitamin A biofortified corn varieties are created through marker assisted pyramiding strategies of β-*Carotene Hydroxylase, Lycopene-ε-Cyclase* and *Opaque2* genes through backcrosses and selection breeding [86].

Performing the same feat achieved in corn in other crops was more challenging. Galili and Amir [87] compiled a review of studies that involved seed protein improvement by genetically manipulating amino acid contents right from the discovery of *Opaque-2* up till 2013. The review showed that apart from maize, classical genetics rarely produced commercially viable varieties in other crops, hence the transgenic breeding methods were engaged. To date, only two genetically modified (GM) events have been commercialized in cereal crops to modify AA-traits [88]. These are the dapA-gene (*Corynebacterium glutamicum*), which increases free-Lys content and the cor-dapA gene, which encodes the enzyme that catalyzes the first reaction in the Lys biosynthetic pathway [88]. However, the introgression of foreign genes affects the acceptability of GM crops for cultivation due to the possibility of potential toxicity, allergenic effects, genetic drifts to other crops, and environmental hazards.

Within the last decade, alternative techniques have been developed that makes it possible to avoid the introgression of foreign genes and transgenic GM crops including e.g., cisgenesis, intragenesis and genome editing [88, 89]. Genome editing techniques include engineered endonucleases/meganucleases (EMNs), zinc-finger nucleases (ZFNs), TAL effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) [90–92]. Genome editing has been used in maize and soybeans to target the gene that encodes enzymes that catalyzes the first step in the biosynthesis pathway of some EAAs [90, 92]. However, research studies that incorporate these strategies for seed dietary proteins in seeds are still sparse, though there are reviews on the possible application of these technologies for improving other seed quality traits [93]. There are prospects of generating populations for improving seed proteins without transgenic breeding with these technologies.

5. Future research gaps

With the current global awareness, the development of best possible organoleptic and nutritious qualities of food from sustainable plant proteins to feed the

ever-increasing global population will continue despite the enormous knowledge been generated in the last decade (**Table 4**). The data on the knowledge base confirms the assertions that opportunities exist to overcome technology obstacles and nutrition and safety challenges in further developing the alternative plant-based protein markets from grain crop sources [94].

The health products from plant-based proteins are the key selling points for the emerging consumer shift, because it is where significant growth in research and innovations is happening (**Table 4**). In this case, the discovery of bioactive peptides is a critical research area in the dynamics of peptide sources, sequences, structure, networks, and functionality in relation to specific health issues or even emergencies like the SARS-CoV-2 pandemic [95]. A call for bioactive peptides in PubMed for COVID generated 723 reference listings in January 2021. Secondly, the standardization of protein bio-availability is also an active area of knowledge generation that falls under the seed protein quality testing for diversification of protein sources. Under methods of production, the evaluation of functionality has become a space for multiplied research activities as the industry continues to innovate formulations. Composite EAA strategies continues to generate new nutraceuticals, which is exposing new knowledge gaps for the standardization of protocols for protein bio-availability measurements (PDCAAS in the US and PER in Europe) to DIAAS for global regulatory compliance with bio-availability measurements [96]. Thirdly, industry acceptance thrives on organoleptic acceptance, texture and taste of ever-increasing formulations of animal protein plant analogs, thus standardizing sensory evaluation techniques requires continuing research efforts as products are formulated. Lastly, the field of genetics and breeding of plant protein crops is a space

Research themes	2010	2012	2014	2016	2018	2020
1. Crop source diversification • Protein and EAA contents/ Bioavailability (PDCAAS/ DIAAS) evaluation	24	36	38	43	53	67
2. Health and functional food development • Nutraceuticals, functional foods, bioactive peptides	508	762	1120	1582	2107	2903
3. Product improvement through processing for functionality • Functionality, Physico-chemical properties, meat and diary substitutes (analogs), composite foods strategies and EAA bio-availability	1883	2284	2495	2484	2708	1893
4. Crop genetics • Multi-omics platforms for genetic networks and trait association analysis, plant breeding strategies for crop variety development for specific protein food products	1188	1494	1630	1650	1665	1252

Table 4.
References from calls on PubMed and associated libraries with various research themes and call terms including "plant-based seed proteins". Calls were restricted to each year of 2010 to 2020. Other references were accessed from associated journals within PubMed and associated libraries.

where the knowledge gap remains very wide. Being at the base of the value chain for plant protein innovations, genetics promises future gains for the protein production systems. Besides genetic engineering techniques, one prominent approach in the future of advancing plant-based food production systems is the emerging breeding technique that combines the use of artificial intelligence (AI) individual seed selection, cloud-based omics diversity databases and machine learning algorithms to identify and develop situation specific protein varieties in a short time. With the cloud computing support and robust prediction algorithms, the capacity to analyze large genomic and phenotypic datasets enables scientists and breeders to easily associate genomic sequences with beneficial traits. The outlook for the development of dietary protein seeds with these advances promises the possibility of personalized nutrition, the possibility of cost-effective trait development, accelerated breeding cycles, and better management of environmental resources for better nutrition.

Moreover, with the expanding knowledge in plant proteins will come the need for environmental datasets across the value chain from field to the table. Dynamic datasets on environmental footprints will continue to be in demand to settle contentions of the animal protein and the emerging plant protein industries and strike the balance in the industry.

6. Conclusion

The combination of various factors that compels research and innovations in the field of plant-based dietary proteins include the realities of proven nutritional and health benefits and its benefit in promoting ecologically sustainable food production systems. Research efforts in this field have generated a body of knowledge that requires to be updated and consolidated on a steady basis given the fast pace of research activities and volume of scientific publications. This review provides a modest update on the place of seeds (grains) in the development of plant-based protein foods. The review focused on PubMed library and other literature resources to probe the subjects of crop sources of dietary proteins, the state of functional and health benefits from seed-based dietary proteins, functionality manipulations to achieve animal protein analogs, and the state of crop genetics in the improvement of grain-based dietary proteins. The review illuminates the enormity of information and the fast pace of knowledge generation in three key research themes which in turn creates new knowledge gaps that draws from the other research themes. These key knowledge areas are: (1) Continuous generation of health-related functional foods and nutraceuticals from grain-based proteins. The development of bioactive peptides for specific health issues at specific personal physiological conditions will continue to be an active research area with potentials for advancing nutrigenomics sciences in the near future. (2) Plant protein quality research in terms of bioavailability and functionality of the ever-increasing fortification strategies. The pace of identification and formulation of plant protein foods creates knowledge gaps that demands research attention for the harmonization of regulatory policies in the various global jurisdictions for promoting the seed protein innovation markets. (3) At the base of the value chain of plant-based proteins is the genetics and breeding of targeted dietary protein and nutritional traits. The future will see the application of advancing omics tools, databases, and networks to the breeding of new varieties in record time for the emerging plant-based protein food systems.

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Section 2

Proteins Functionality

Chapter 6

Modification of Legume Proteins for Improved Functionality

Asli Can Karaca

Abstract

Recent studies have indicated that legume proteins can be potentially used as an alternative to animal-derived protein ingredients for many food and biomaterial applications, however some modifications may be first required to improve their functionality since they show relatively lower solubility and functional properties compared to commonly used animal-based proteins. A variety of physical, chemical or biological processes can be used to achieve these modifications in structural, physicochemical, and functional properties of legume proteins. The aim of this chapter was to review the most recent studies focusing on modification of structural properties and improvement of functionality of legume proteins. Effects of processing conditions on protein functionality were discussed. Special emphasis was given to the structure–function mechanisms behind these changes. Since the performance of modified legume proteins has been shown to depend on a variety of factors; parameters used in the modification process have to be optimized to achieve the desired level of improvement in legume protein functionality. Each modification method has been indicated to have its own advantages and limitations in terms of performance and applicability in different food matrices. Further studies are required to investigate the interactions of modified legume proteins with other food components during food processing and storage. Furthermore, additional research on the effects of modification treatments on flavor profile and nutritional properties of legume proteins is needed as well.

Keywords: legume protein, modification, structure, functional properties, physicochemical properties

1. Introduction

Legumes belong to the *Fabaceae* family plants and involve different species including peas, beans, lentils, and chickpeas [1]. Legume proteins have gained the interest of food industry due to their low cost, low risk of allergy, good functional and nutritional properties [2]. Their potential use as ingredients in a variety of food applications has been widely investigated recently. However, due to their relatively lower solubility and performance compared to animal-derived proteins, some modifications may be required to obtain optimum functionality. There are many different techniques available for altering the structural properties and improvement of functionality of legume proteins. Each method has its unique advantages and limitations while selection of the most suitable method of modification depends on various factors including protein source and composition, processing conditions, feasibility, cost, and the application area of the modified protein.

Recently, Sharif et al. [3] reviewed the modification methods applied to legume proteins for improvement of emulsifying properties for encapsulation applications. Ge et al. [4] discussed the modifications applied specifically to pea protein for improved functionality. This chapter presents an overview from a broader perspective of the most commonly used methods for modification of functional properties of legume proteins, with a major focus on the most recent studies.

2. Physical modifications

Functional properties of legume proteins can be modified by application of a variety of physical methods including thermal treatment, ultrasonication, and high pressure. A brief summary of the findings of recent studies focusing on investigation of the effects of physical methods on functionality of legume proteins is presented in **Table 1**.

2.1 Thermal treatment

Thermal treatment is commonly applied to legume proteins for modification of structure and functionality. Tang et al. [5] applied heat treatment to kidney, red bean and mung bean protein isolates at 95 °C for 30 min and studied its effects on structural properties and functionality of the proteins. The authors observed heat-induced protein denaturation which was evident by the modifications in the secondary and tertiary protein structures. Heat treatment was indicated to increase the surface hydrophobicity of bean proteins due to unraveling of previously buried hydrophobic moieties. Although the authors observed protein denaturation and significant increase in hydrophobicity, solubility of heat-treated bean proteins was reported to increase as well. This finding was attributed to the possible increase in charged residues on protein surface due to denaturation and partial unfolding of the molecule. Emulsifying activity of heat-treated bean proteins was also reported to increase compared to the native proteins due to increased hydrophobicity and solubility after heat treatment. Peng et al. [8] investigated the effect of heat treatment (95 °C, 30 min) on surface hydrophobicity, interfacial properties, emulsifying activity and stability of pea proteins. It was reported that heat treatment increased surface hydrophobicity due to unraveling of protein structure and exposure of hydrophobic groups buried inside the molecule. Application of heat treatment was reported to result in slightly reduced interfacial tension and significantly higher percentage of adsorbed proteins at the interface compared to the native protein. Heat-treated pea protein was able to form emulsions with significantly smaller droplets and higher stability against creaming due to higher adsorption of proteins at the oil–water interface. Chao and Aluko [9] applied heat treatment to pea protein isolate at a wider temperature range (50–100 °C, 30 min) for modification of emulsifying and foaming properties. The authors observed changes in protein conformation and increased hydrophobicity which were attributed to protein unfolding. Those changes in protein structure were reported to lead to protein aggregation induced by thermal treatment up to 100 °C. Emulsion formation and stabilization by pea proteins were reported to improve at pH 7.0, which was indicated by reduced oil droplet size compared to the native protein. However, average oil droplet size of heat treated pea protein-stabilized emulsions were reported to be larger than that of the native protein-stabilized emulsions at pH 5.0. Foaming properties of pea protein were observed to be negatively affected by heat treatment regardless of the pH applied. In another recent study, Bühler et al. [7] applied dry heating to faba bean protein concentrate in an air oven (75–175 °C, 1 h) for modification of water holding capacity. The authors used

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Thermal treatment	Red kidney bean, red bean and mung bean proteins	95 °C, 30 min	Improved solubility and emulsifying activity	[5]
	Red kidney bean protein	95 °C, 15–120 min	Improved solubility, emulsifying and foaming activities with moderate heating (15–30 min)	[6]
	Faba bean protein	75–175 °C, 60 min	Increased water-holding capacity, decreased solubility	[7]
	Pea protein	95 °C, 30 min	Higher adsorption at the oil–water interface, improved creaming stability, increased viscosity	[8]
	Pea protein	50–100 °C, 30 min, pH 3.0–7.0	Improved emulsifying activity at pH 7.0, decreased foaming properties regardless of pH	[9]
Ultrasonication	Black bean protein	20 kHz, 150–450 W, 12–24 min	Improved solubility	[10]
	Soybean β-conglycinin and glycinin	20 kHz, 400 W, 5–40 min	Improved solubility, emulsifying activity, and stability	[11]
	Faba bean protein	20 kHz, 50–75% amplitude, 15–30 min	Higher adsorption at the oil–water interface, improved foaming properties	[12]
	Pea protein	20 kHz, 30–90% amplitude, 30 min	Improved foaming properties	[13]
	Chickpea protein	20 kHz, 300 W, 5–20 min	Improved solubility, emulsifying, foaming, water holding and gelling properties	[14]
High pressure	Kidney bean protein	200–600 MPa, 15 min	Improved water holding capacity, foaming, and emulsifying properties, increased viscosity	[15]
	Yellow field pea protein	200–600 MPa, 5 min	Improved emulsifying and foaming properties	[16]
	Lentil protein	25–150 MPa	Improved solubility, emulsifying and foaming properties with increasing pressure up to 100 MPa, improved gelling properties at 50–150 MPa	[17]
Other techniques	Pea protein	Controlled shear at 100–1500 s^{-1}	Improved solubility and heat stability	[18]
	Pea protein	Cold atmospheric pressure plasma	Increased water and fat binding capacities, improved solubility	[19]
	Grass pea protein	Cold atmospheric pressure plasma	Higher adsorption at the oil–water interface, improved emulsifying properties	[20]

Table 1.
Summary of recent studies on physical modification of legume proteins for improved functionality.

soy protein concentrate as a reference. Heat treatment was reported to induce partial denaturation, which in turn increased hydrophobicity and resulted in decreased protein solubility. On the contrary, water holding capacity of faba bean protein was observed to increase and became comparable to soy protein.

2.2 Ultrasonication

Ultrasonication has been recently investigated as a novel tool for modifying the structure and improving functionality of legume proteins. Gharibzahedi and Smith [21] recently reviewed the current applications and challenges of this technology for legume proteins. Jiang et al. [10] applied low-frequency (20 kHz) ultrasonication to black bean protein isolate. Primary structure of black bean protein was reported to remain unchanged after the ultrasonication treatment indicated by the electrophoresis profile. On the other hand, secondary structure of black bean protein was observed to change after ultrasonication where the proportion of α-helix decreased and β-sheet increased. Ultrasonication-related alterations in tertiary structure were monitored by the changes in fluorescence spectra. Average particle size of ultrasound-treated black bean protein was reported to be larger compared to that of native protein which was attributed to formation of aggregates. Net surface charge of ultrasonicated black bean protein was observed to change depending on the power applied. Surface hydrophobicity of black bean protein was also reported to increase after the ultrasonication treatment. Similar to heat treatment applications, ultrasonication is indicated to result in unfolding of the protein molecule up to a certain degree and increase hydrophobicity. However, surface hydrophobicity was reported to decrease with a further increase in the ultrasonication power due to aggregation of protein molecules which in turn enclose the hydrophobic sections of the protein. Ultrasonication treatment was reported to result in an increase in solubility of black bean protein, directly related to ultrasonication power and treatment time which was based on increased interaction between protein molecules and water. The authors concluded that the modifications on protein functionality could be optimized based on the ultrasonication conditions. Martinez-Velasco et al. [12] studied the effect of ultrasound treatment on surface characteristics and foaming properties of faba bean protein isolate. The authors optimized the parameters of ultrasonication treatment and reported that an amplitude of 73% and duration of 17 min resulted in lower interfacial tension, higher solubility, higher adsorption at the interface, smaller bubble diameter, and higher foam stability. In a similar study, Xiong et al. [13] investigated the effect of ultrasound treatment on structural characteristics and foaming ability and stability of pea protein isolate. Ultrasonication was reported to induce unfolding of the protein molecule up to a certain degree and hence increased hydrophobicity. These changes combined with decreased particle size were reported to result in decreased surface tension, improved foaming ability and stability after the treatment. In a recent study, Wang et al. [14] applied ultrasound treatment to chickpea protein isolate and investigated the changes in interfacial, physicochemical, and gelling properties. The authors observed increase in solubility, foaming capacity, emulsifying activity, and gel strength as a result of the ultrasonication treatment. Improved gelling properties of the ultrasonicated chickpea protein were attributed to the changes observed in hydrophobicity and particle size as a result of the ultrasonication treatment.

2.3 High pressure

Potential of high pressure application to modify the structure and functionality of legume proteins has been recently investigated by various researchers. Chao et al. [16]

applied high hydrostatic pressure (200–600 MPa) to yellow field pea protein isolate and determined its effects on physicochemical characteristics and functional properties of the protein. Gel electrophoresis patterns were observed to indicate aggregation due to increased interaction between protein molecules after high pressure application. High pressure treatment was reported to induce changes in the tertiary structure identified by fluorescence spectroscopy. The extent of structural changes depended on the level of pressure applied. The highest level of denaturation and unfolding of the molecule was observed at 600 MPa pressure. Despite those changes, protein solubility was found to remain unchanged. At the same time, emulsifying properties of yellow field pea protein were reported to be improved after the high hydrostatic pressure treatment. Mean droplet size of emulsions stabilized by high pressure-treated yellow field pea protein was reported to be significantly smaller compared to that of emulsions stabilized by untreated protein. Formation of smaller oil droplets was attributed to increased interactions between oil droplets and unraveled hydrophobic groups of high pressure-treated protein. Effect of high pressure treatment on emulsion stability was found to change depending on protein concentration and pH. Specifically, at relatively lower protein concentrations, pH 3.0 and 5.0, emulsion stability was reported to decrease as a result of high pressure treatment which was attributed to protein aggregation decreasing the ability of the protein to form strong interfacial membranes. On the other hand, at pH 7.0, stability of pressure-treated protein-stabilized emulsions was reported to be higher compared to that of untreated protein due to increased surface charge. Foaming properties of yellow field pea protein isolate were found to be improved with high pressure treatment at all protein concentrations and pH values studied. Sim et al. [22] compared the efficacy of high pressure treatment (250–550 MPa) with heat treatment (95 °C, 15 min) with respect to gel forming properties of pea protein concentrate. The authors indicated that high pressure treatment changed the tertiary and quaternary structure of pea protein which resulted in protein denaturation, aggregation, and network formation. The extent of these changes increased with increasing pressure level. Pea protein subjected to heat treatment formed a gel at a lower concentration (12 g/100 g) compared to the pea protein subjected to high pressure at 250 MPa for 15 min (16 g/100 g). Moreover, heat-treated pea protein was reported to show greater gel strength compared to high pressure-treated protein. Ahmed et al. [15] applied high pressure treatment (200–600 MPa) to kidney bean protein isolate and determined the changes in protein structure and functionality. Investigation of thermal properties of kidney bean protein revealed that high pressure treatment resulted in unraveling of protein structure. Protein unfolding and changes in tertiary structure due to high pressure application were also verified by the shifts observed in Fourier transfer infrared (FTIR) spectroscopy profile. The magnitude of those changes increased with increasing pressure level. High pressure-treated pea protein was reported to show higher thermal denaturation temperature, improved water holding capacity, foaming, and emulsifying properties when the treatment was applied at above 600 MPa. In a recent study, Saricaoglu [17] applied high pressure (25–150 MPa) homogenization for improving functional properties of lentil protein isolate. The author reported that an increase in pressure from 50 to 150 MPa resulted in unraveling of the protein structure and improved solubility, emulsifying, and foaming properties of lentil protein. However, high pressure-treated lentil protein was observed to form weak gel-like structures.

2.4 Other techniques

Other physical techniques including cold plasma and controlled shear have also been applied to legume proteins for modification of their structural and functional properties. In a recent study, Mehr and Koocheki [20] applied atmospheric cold

plasma to Grass pea protein isolate and monitored the changes in protein structure and emulsifying properties. The authors reported that the extent of changes in protein structure depended on the level of voltage applied and duration of application. An initial increase in voltage and treatment time was indicated to result in an increase in the content of carbonyl groups which was determined to monitor the interaction between the amino acid side chains and reactive chemical species of plasma. On the other hand, the amount of free sulfhydryl groups was observed to decrease with the plasma treatment which indicated the structural modification of Grass pea protein. At the optimized treatment conditions globulins were dissociated, increasing the absorption rate of protein into the oil–water interface. Cold plasma treatment applied was indicated to alter the protein structure on secondary and tertiary levels. Ability of the plasma treated protein to decrease the interfacial tension was reported to be affected by the treatment conditions in such a way that lower voltage values resulted in lower interfacial tension. On the contrary, emulsion stabilized with the protein treated with higher voltage was observed to have smaller oil droplets and show higher stability against creaming. Bussler et al. [19] compared the efficiency of thermal treatment with cold plasma treatment for improvement of functionality of grain pea proteins. Plasma treatment was reported to induce changes in the tertiary or quaternary structure which in turn resulted in increased solubility, water and fat binding capacities. It was concluded that the plasma treatment applied could potentially be used as an alternative non-thermal method for improving protein functionality. In a recent study, Bogahawaththa et al. [18] applied controlled shear (100 or 1500 s^{-1}) to pea protein isolate and determined the changes in protein solubility and heat stability during heating at 90 °C for 5 min. It was reported that pea protein subjected to shear at 1500 s^{-1} showed significantly higher solubility and heat stability compared to the protein subjected to shear at 100 s^{-1} which was based on the changes in secondary structure and formation of soluble hydrophobic aggregates at high shear.

3. Chemical modifications

Structure and functionality of legume proteins are also modified by application of a variety of chemical methods including attachment of various molecules to protein structure via different pathways. Some recent reports on chemical methods applied to legume proteins for improved functionality are summarized in **Table 2**.

3.1 Attachment of low molecular weight molecules

One of the most commonly applied methods for improving protein functionality is attachment of low molecular weight molecules to protein structure via different chemical pathways [3]. Yin et al. [33] reported that acetylation and succinylation treatments induced changes in the secondary and/or tertiary structures of kidney bean protein isolate and resulted in a decrease in isoelectric point and net surface charge at pH 7.0. Succinylation was reported to decrease surface hydrophobicity of kidney bean protein while acetylation increased hydrophobicity. Charoensuk et al. [25] indicated that the effect of succinylation treatment on mung bean protein isolate depended on the ratio of succinic anhydride to protein. A decrease in isoelectric point and net surface charge at pH 7.0 was observed as a result of succinylation whereas the amount of free sulfhydryl groups was not affected. Improved emulsifying activity after succinylation treatment was attributed to increased flexibility of succinylated protein. Shah et al. [27] hydrophobically modified pea

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Attachment of low molecular weight molecules	Chickpea protein	Acetylation (6–49%)	Higher solubility at pH > 8.0. Lower solubility at pH 2.0–7.0. Improved water and oil absorption capacities, higher emulsion capacity, lower emulsion stability	[23]
	Lentil protein	Succinylation (58–90%)	Higher solubility at pH > 4.0. Lower solubility at lower pH. Increased water absorption capacity, viscosity, emulsifying activity and stability. Decreased oil absorption capacity, foaming capacity and stability.	[24]
	Mung bean protein	Succinylation (9–40%)	Improved solubility and emulsifying activity	[25]
	Black kidney bean protein	PEGylation	Higher gelling strength and shorter gelling time	[26]
	Pea protein	Hydrophobical modifications by *N*-substitutions	Improved solubility, foaming capacity, stability, emulsion stability, and water holding capacity	[27]
Attachment of high molecular weight molecules	Mung bean protein	Ultrasound pre-treatment and Maillard reaction with glucose	Improved solubility, emulsifying activity and stability	[28]
	Mung bean protein	Maillard reaction with dextran	Improved solubility, emulsifying activity and stability	[29]
	Black bean protein isolate	Ultrasound pre-treatment and Maillard reaction with glucose	Improved solubility and emulsifying properties	[30]
	Pea protein	Maillard reaction with gum Arabic	Improved solubility and emulsifying properties	[31]
	Soya bean protein	Maillard reaction with maltodextrin	Improved solubility, decreased gel strength and water holding capacity	[32]

Table 2.
Summary of recent studies on chemical modification of legume proteins for improved functionality.

protein by *N*-substitutions using different reactants. Hydrophobical modifications applied induced changes in the secondary structure of pea protein which were observed with FTIR spectroscopy. Alterations in secondary structure were attributed to increased negative charge. Changes in thermal profile were based on partial denaturation of protein and aggregation. Solubility and water holding capacity of modified pea protein were observed to increase due to increased negative charge. Emulsion stability index was reported to increase as a result of increased charge, solubility and addition of hydrophobic groups to the protein molecule. Hydrophobically modified pea protein with improved functionality was indicated to have a potential to be used as egg replacers in cake formulations.

3.2 Maillard reaction

Forming protein-polysaccharide based conjugates through glycation or Maillard reaction has been indicated as a promising method for modification of protein functionality [34]. Zhou et al. [29] formed conjugates between mung bean protein isolate and dextran at 80–90 °C for changing durations of 1–6 h. Electrophoretic profile of mung bean protein showed that both vicilin and legumin subunits participated in the Maillard reaction. Conjugation was indicated to alter the secondary structure, decreasing the α-helix content due to heat-induced unfolding of the molecule and attachment of dextran. Fluorescence spectra were used as an indicator to monitor the changes in tertiary structure. The authors proposed that conjugation induced unfolding of mung bean protein up to a certain extent and increased the flexibility of protein structure. Solubility of the conjugates formed at 2–3 h was reported to be increased compared to mung bean protein due to the increased number of hydrophilic moieties introduced by the grafted dextran. However, a slight decrease in solubility was observed with increasing graft time due to formation of insoluble protein aggregates. Emulsifying activity and stability indices of conjugates followed a similar trend and first increased compared to the native mung bean protein and then decreased with increasing graft time. Initial increase in emulsifying activity and stability indices was attributed to improved solubility and flexibility due to conjugation. The possible mechanism behind impaired emulsifying properties observed with increasing graft time was explained by heat induced protein aggregation and reduction of interfacial activity of mung bean protein with increased attachment of dextran. The authors reported that conjugates formed at 80 °C with lower glycosylation degrees and browning showed better functionality compared to the conjugates formed at 90 °C.

Wang et al. [28] investigated the effect of ultrasound treatment on conjugation of mung bean protein isolate and glucose. Similar to the study of Zhou et al. [29], the authors reported that Maillard reaction resulted in changes in the secondary structure of mung bean protein. Furthermore, ultrasound-treated conjugates were reported to have a less compact tertiary structure compared to the heat-treated conjugates and the native protein. Application of ultrasound treatment in Maillard reaction was indicated to form conjugates with a higher degree of glycosylation and improved solubility. Higher solubility observed in ultrasound-treated conjugates was attributed to two factors: breaking of insoluble aggregates and addition of more hydrophilic groups due to enhanced conjugation with the ultrasonication treatment. Similarly, ultrasound-treated conjugates showed better emulsifying activity and stability compared to heat-treated conjugates due to dispersion of aggregates and improved mobility of the protein molecule. Jin et al. [30] also investigated the effect of ultrasound treatment on conjugation of black bean protein isolate and glucose via Maillard reaction. Ultrasound treatment was reported the increase the reaction rate indicated with a higher degree of glycation at a shorter time. FTIR profile of the samples indicated that ultrasound-treated conjugates lost more ordered secondary structure (α-helix and β-sheet content) compared to the heat-treated conjugates. Alterations in protein structure resulting in increased unordered structure content were reported to improve the flexibility of the molecule and hence, emulsifying properties. Changes in fluorescence spectra indicated that the Maillard reaction resulted in alterations in the tertiary structure and ultrasonication treatment further increased the extent of these changes. Moreover, ultrasound-treated conjugates were reported to show higher surface hydrophobicity, improved solubility, emulsifying activity and stability indices compared to the heat-treated conjugates and the native protein. In another recent study, Zha et al. [31] formed conjugates between pea protein and gum Arabic and monitored the changes in functional properties

and flavor profile of pea protein. Pea protein-gum Arabic conjugates were reported to show improved solubility compared to the native pea protein. Incubation time was indicated as an important factor affecting the solubility of conjugates. Emulsion forming and stabilizing ability was determined by measuring droplet size. Emulsions stabilized by pea protein-gum Arabic conjugates were reported to have smaller droplets compared to emulsions stabilized by pea protein. Conjugate-stabilized emulsions showed the highest stability against environmental factors including temperature, pH and ionic strength when incubation time was kept at 3 days. The undesired (beany or grassy) flavor markers in pea protein were reported to decrease significantly after 1 day of incubation. Increasing the incubation time was reported to improve the flavor profile of the conjugates further.

4. Biological modifications

Functional properties of legume proteins can be improved via various biological methods including enzymatic hydrolysis, cross linking and fermentation. A brief summary of the findings of recent studies focusing on biological modification of legume proteins is presented in **Table 3**.

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Hydrolysis	Lentil protein	Hydrolysis with trypsin (4–20% DH[1])	Decreased interfacial tension. Decreased emulsifying activity and stability	[35]
	Chickpea protein	Hydrolysis with Flavourzyme (1–10% DH)	Improved solubility, oil absorption and foaming capacities. Decreased emulsifying activity	[36]
	Chickpea protein	Hydrolysis with Alcalase (4–15% DH)	Improved solubility. Decreased interfacial tension. Decreased emulsifying activity and stability	[37]
	Chickpea protein	Hydrolysis with Alcalase and Flavourzyme (12–50% DH)	Improved solubility. Hydrolysates with lower DH showed better emulsion properties	[38]
	Bambara bean protein	Hydrolysis with pancreatin (5–34% DH)	Improved solubility and oil-holding capacity. Decreased emulsifying activity	[39]
	Black bean protein	Hydrolysis with pepsin and Alcalase (24–28% DH)	Alcalase hydrolysates showed higher emulsion stability	[40]
	Faba bean protein	Hydrolysis with different proteases (2–16% DH)	Improved solubility, foaming capacity, oil holding capacity	[41]
	Pea protein	Hydrolysis with trypsin (2–4% DH)	Improved solubility. Samples at 2% DH showed better emulsion properties	[42]
	Pea protein	Hydrolysis with different proteases (2–10% DH)	Improved solubility. Hydrolysates obtained with trypsin showed the highest foaming and emulsifying capacities	[43]

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Cross-linking with transglutaminase	Kidney bean protein	5 U enzyme/g protein, 37 °C, 0–240 min	Decreased solubility, emulsifying activity and stability	[44]
	Pea protein	10.5% protein, 0.3 M NaCl, 10 U enzyme, 40 °C, 30 min	Higher gel strength and more elastic gel formation	[45]
	Pea protein	17–23% protein, 5–7 U enzyme/g protein, 40–90 °C, 30–60 min	Higher gel strength and flexibility of the gel network	[46]
	Chickpea protein	200 U enzyme/g of protein, 37 °C, 60 min	Formation of a gel-like emulsion, improved emulsion stability	[47]
Fermentation	Protein-enriched pea flour	*Lactobacillus plantarum* NRRL B-4496, 7 log CFU/g flour, 32 °C, 11 h	Improved foaming capacity, emulsion stability, and oil-holding capacity. Decreased foam stability and emulsifying activity	[48]
	Protein-enriched pea flour	*Aspergillus niger* NRRL 334, *Aspergillus oryzae* NRRL 5590, 7 log CFU/g flour, 40 °C, 6 h	Decreased solubility and foaming properties. No significant changes in emulsifying properties. Improved water and oil binding properties	[49]
	Lupin protein	Fermentation with eight different microorganisms, 7 log CFU/g protein, 24 h	No significant changes in foaming activity and emulsifying capacity. Decreased solubility at pH 7.0	[50]

[1]*DH: degree of hydrolysis.*

Table 3.
Summary of recent studies on biological modification of legume proteins for improved functionality.

4.1 Enzymatic hydrolysis

Application of enzymatic hydrolysis for modification of structural properties and improving functionality of legume proteins has been widely studied recently. Klost and Drusch [42] applied enzymatic hydrolysis for modification of solubility and interfacial properties of pea protein. Hydrolysis with trypsin up to 4% degree was reported to increase solubility from 30% to 60% at pH 4.0–6.0 due to the increased amount of terminal COO^- and $NH3^+$ groups. On the other hand, net surface charge and solubility were reported to decrease at pH 3.0 and 7.0 due to exposure of previously buried hydrophobic moieties leading to aggregation. Pea protein hydrolysates were observed to form emulsions with wider oil droplet size distributions which were not stable against creaming as a result of decreased net charge and lack of repulsion. Enzymatic hydrolysis was reported to positively affect the strength of interfacial films formed. In a recent study 11 different proteolytic enzymes were used for hydrolyzing pea protein isolate for improved functional and sensory properties [43]. Solubility of most of the hydrolysates at pH 4.5 was reported to be improved with decreasing peptide size and release of hydrophilic amino acids with hydrolysis. Among the hydrolysates studied, Esperase hydrolysates were reported to show the highest protein solubility whereas the highest foaming and emulsifying capacities were observed in Trypsin hydrolysates.

All hydrolysates were observed to have improved foaming capacity and stability compared to the native protein which was based on decreased peptide size and modification of surface hydrophobicity with hydrolysis. Among the sensory attributes evaluated, only bitterness was reported to change significantly after hydrolysis. Some of the hydrolysates were reported to have lower bitterness scores than that of the native protein. However, increased degree of hydrolysis resulted in increased bitterness. In another recent study, four different proteases were for used for investigation of combined effects of enzymatic hydrolysis and ultrafiltration treatment on faba bean protein functionality [41]. It was reported that hydrolysis with pepsin resulted in significant increases in solubility, foaming and oil holding capacities of faba bean protein. Fractionation with ultrafiltration was observed to allow for further improvements in foaming, oil holding and emulsifying capacities of the peptides obtained.

4.2 Cross-linking

Enzymatic cross-linking with transglutaminase is another biological approach used for improving protein functionality. Tang et al. [44] studied the effect of cross-linking on kidney bean protein isolate. The authors observed unfolding of the vicilin units and formation of higher molecular weight oligomers. Thermal stability of vicilin-rich kidney bean protein was reported to increase after the cross-linking treatment. However, the authors observed gradual decrease in solubility and emulsifying properties with increasing incubation time. Moreno et al. [46] compared the efficiency of cross-linking and thermal processing in gelation of pea protein. The main aim of the cross-linking treatment was to obtain improved gelling properties for various meat and seafood applications. It was reported that cross-linking resulted in polymerization of vicilin and legumin subunits forming new intermolecular protein complexes indicated by alterations in protein structure. Transglutaminase treatment was reported to improve conformational stability and flexibility of the gel network. Cross-linking with transglutaminase is generally applied to legume proteins for improving gelling properties. However, Glusac et al. [47] studied its effects on characteristics of emulsions stabilized by chickpea protein. Cross-linking treatment was reported to increase mean droplet size of the emulsion compared to the native protein and formed a gel-like structure. Emulsions were observed over a month and cross-linked chickpea protein-stabilized emulsions were indicated to show higher stability against phase separation compared to the emulsions stabilized by native protein.

4.3 Fermentation

Although fermentation is a traditional process, its application for improving functional, nutritional and sensory properties of legume proteins and protein-rich flours has gained interest in the recent years. Cabuk et al. [48] investigated the effect of fermentation on properties of pea protein-enriched flour (~40 g/100 g protein). Fermentation was conducted for 11 h where the degree of hydrolysis was reported to reach 13.5%. Net surface charge at pH 4.0 was reported to increase at 1 h of fermentation and then decreased. Net surface charge at pH 7.0 and surface hydrophobicity at pH 4.0 were reported to increase. Solubility at pH 7.0 was reported to decrease from ~43% to 36% after 11 h of fermentation. The highest foaming capacity was reported for pea protein-enriched flour fermented for 5 h at pH 4.0. Emulsifying activity of pea protein-enriched flour was observed to decrease after 5 h whereas emulsion stability increased. It was concluded that functionality of fermented protein-enriched flours can be optimized depending on fermentation

conditions. In a follow-up study, fermentation was conducted with two different *Aspergillus* strains for 6 h and the degree of hydrolysis was reported to reach 10–11% [49]. The authors observed an increase in surface charge with increasing fermentation time whereas surface hydrophobicity was reported to decrease. Fermentation was indicated to result in negative effects on solubility and foaming properties where emulsifying properties were reported to remain unchanged. Decrease in solubility was attributed to increased protein–protein interactions and aggregation. On the other hand, water and oil binding properties were observed to be improved after fermentation. In another recent study, Schlegel et al. [50] used eight different microorganisms for fermenting lupin protein isolate. Fermentation was reported to result in no significant difference in solubility of lupin protein at pH 4.0; however, solubility at pH 7.0 decreased from ~64% to <42%. Emulsifying capacity of lupin protein was not affected by fermentation. On the other hand, foaming activity of fermented lupin protein was reported to be higher than that of native protein. Among the microorganisms studied, only two of them resulted in improved emulsifying capacity in the fermented lupin protein compared to the native protein. All microorganisms used for fermentation were found to decrease the bitterness score of lupin protein. *Lactobacillus brevis* was reported to be the most effective microorganism for improving the sensory profile as it was noted to decrease the intensity of undesired flavor markers.

5. Conclusion

A variety of physical, chemical and biological methods can be applied to legume proteins for modification of their structural and functional properties. Each method has its own advantages and limitations in terms of performance and applicability in food systems. Many factors affect the performance of the modified legume proteins: protein structure and composition, modification method and processing conditions applied, and extrinsic factors such as pH, ionic strength, and temperature. Modification method has to be optimized in terms of processing parameters to obtain the desired level of functionality for the legume protein studied. Complexity of the food matrix is also an important factor affecting the end product performance of modified legume proteins. Further research has to focus on interactions of modified legume proteins with other food components during processing and storage. More studies are required to investigate the effects of modification treatments on flavor profile and nutritional properties of legume proteins.

Conflict of interest

The author declares no conflicts of interest.

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Chapter 7

Pea Seed Proteins: A Nutritional and Nutraceutical Update

Sandeep Kaur Dhaliwal, Pooja Salaria and Prashant Kaushik

Abstract

Grain legumes are well known as staple sources of soluble protein worldwide. Pea is essentially the most quickly growing crop for immediate human consumption and has the potential for higher effect as being a protein supply for foods processing apps. Pea seeds are an essential source of plant-based proteins. The better acceptance of pea protein-rich food is due to pea manifold attributes, excellent functional qualities, high vitamin value, accessibility, and comparatively small cost. Pea proteins are not merely nutritional amino acids but are an indispensable source of bioactive peptides that offer health benefits. This chapter focuses on the present information of isolation methods, extraction, and of seed proteins in pea. Overall, we believe that analogous research and advancement on pea proteins would be required for further more substantial increase in pea protein utilization is envisaged.

Keywords: pea, proteins, food processing, nutritional, health

1. Introduction

Vegetable seed proteins are widely used as ingredients in the food industry. Peas (*Pisum sativum* L.) have grown to be an essential vegetable source of proteins in addition to a likely replacement for soybean [1]. The better acceptance of pea protein-rich food is due to pea manifold attributes, excellent functional qualities in meals programs, high vitamin value, accessibility, and comparatively small cost. Dry peas have 20–30% lysine content. Pea proteins are mainly storage protein composed of albumins and two globulins, legumin and vicilin [2]. Besides, these protein-rich foods are characterized by higher lysine content. The primary pea storage proteins referred to as legumin and pea legumin is hexamer owning a molecular sector (Mw) ~ 320 to 380 kDa. The genuinely bioavailable protein has a pile of easily digested protein, getting a gentle flavour. Unlike extra protein powders with among the top eight allergens as soy, dairy-derived whey, pea protein-rich foods are hypoallergenic; thus it's a great protein alternative for each one of those with and with absolutely no allergies [3]. Pea protein dietary supplements are made in many items. The flexible protein has a packaging that is in unflavored and flavoured blends. Additionally, the pea seeds are loaded with fibre, vitamins, along with micro and macroelements [1].

Proteins obtained from plant sources are expanding ingredient of the marketplace in part due to consumer preferences and their comparatively small cost in contrast to animal-derived proteins [4]. Pea ingredients additionally are attractive to the food market because of their low allergenicity, nutritional value and non-GMO status. While pea does consist of antinutritional components which can inhibit

digestion and may have various prospective deleterious effects pea is still viewed as a too wholesome meal as well as is linked with total health benefits beyond elementary nutrition. The health benefits of pea seed proteins derive primarily from the qualities of starch, vitamins, fibre, protein, phytochemicals and minerals in peas. In this direction, mineral contents and the vitamin of peas may play crucial roles in the protection against deficiency-related diseases, particularly those regarding deficiencies of Folate or Selenium. Peas include a range of phytochemicals previously considered just as antinutritive factors. These contain polyphenolics, in coloured seed layer sorts particularly, that contains anticarcinogenic and antioxidant activity, saponins which might exhibit anticarcinogenic and hypocholesterolemic activity, as well galactose oligosaccharides which might exert beneficial prebiotic consequences within the large intestine [5, 6]. Many strategies for the extraction of protein from pea flours have been reported. Each extraction method might select for different protein sorts which consequently influences the final composition and functionality of the isolated product. In this chapter, we have compiled the information related to pea proteins targeting isolation methods, extraction, and of the seed proteins in pea.

2. Protein content

Protein content in pea lies in a range of 21 to 30 per cent with an average of 23 per cent depending on genotype, growing environment and related factors [6]. The overall phenotypic expression of protein content is a result of environmental as well as genotypic components. The cultivars originating from various geographical areas show a range of protein content levels (**Table 1**). The heritability estimates show that pea protein content and quality is a heritable trait [10, 11], thus target for improvement through selection in breeding programs. Changes in environmental factors such as temperature, rainfall, soil type result in a differential response in performance of pea cultivars; thus multi-location and multi-year data is required for final estimation of protein content [12–14]. Most of the nitrogen supplies during fruit development relies on assimilation after the flowering and only a portion of

Pea seeds	Protein content	Country	Reference
Pisum sativum L. cv. *Ucero*	25.48	Spain	[7]
Pisum sativum L. cv. *Ramrod*	21.17	Spain	[7]
Pisum sativum L. cv. *Agra*	22.90	Spain	[7]
Pisum sativum L. cv. *Maja*	24.21	Serbia	[8]
Pisum sativum L. cv. *Calvedon*	27.70	Serbia	[8]
Pisum sativum L. cv. *Miracle of America*	22.31	Serbia	[8]
Pisum sativum L. cv. *Sprinter*	23.98	Turkey	[6]
Pisum sativum L. cv. *Manuell*	23.26	Turkey	[6]
Pisum sativum L. cv. *Century*	23.9	Canada	[9]
Pisum sativum L. cv. *Trapper*	24.5	Canada	[9]
Pisum sativum L. cv. *Delviche Scotch Green*	24.0	Canada	[9]
Pisum sativum L. cv. *Ceser*	24.9	Canada	[9]
Pisum sativum L. cv. *CD647 4*	24.9	Hungry	[9]

Table 1.
Protein content of famous pea cultivars grown in various parts of the world.

the collection of nitrogen depends on assimilation before flower development [15]. It has been reported that low rainfall and high temperature is positively correlated with high protein content in pea genotypes [13, 16]. A total of 7% high protein content was observed in pea crop raised in dry location than another location having 209 mm higher rainfall indicating role of low rainfall has a significant influence on protein content [13]. However, in another study, there was 1.5% rise in pea protein content between the crop raised in the periodic wilting moisture content of 10 percent versus 26 per cent moisture content at field capacity [17]. In addition, seed yield is known to be negatively correlated with protein content, and these conclusions were made by various independent studies in different years and locations [14, 16, 17]. The dry matter in seed constitutes approximately 50% starch [18, 19]. The dietary fibre and total protein content account for 20 and 24% of the dry matter, respectively. Whereas, 2.5% of dry matter is contributed by lipids [20]. Protein content and starch are highly variable, but other components show little variation [18]. It was found in a study that protein content was negatively correlated with lipid, starch, ash, fibre content and soluble sugar and among these variations in starch content had a significant effect on protein content levels [21]. This study was conducted at four locations in Canada using dehulled pea cultivar, and it was observed that protein content of the cultivar was variable across locations showing levels 14.5%, 18.3%, 24.3%, and 28.5%. The starch synthesis was reported to be a critical factor in determining pea protein content as smooth seeded pea having a higher content of amylopectin and starch showing lower protein levels (23–31%) than wrinkled pea seeds (26–33%) [22]. Recessive gene account for higher protein levels in wrinkled pea seeds.

3. Amino acids

Peas are an excellent source of human nutrition owing to 25% protein in seeds [1], and it has a comparable amino acid (AA) profile to other legumes. Pea protein contains a lesser amount of sulphur amino acids, i.e., methionine and cystine and lower levels of tryptophan AA, whereas high levels of lysine AA [23]. The bioactive peptides of pulses are popularized due to affordable prices when compared with animal protein [24]. During the processing of food, microbial agents or digestive enzymes cause the hydrolysis of large proteins and release bioactive peptides which are usually 3–20 AA long [25]. Nutritional and functional properties food protein are studied using bioactive peptides obtained by hydrolysis through enzymatic action [26]. AA composition of a peptide is the key to its biological activity [24]. Oxidative stress damage in human beings can be prevented by developing nutraceuticals and foods using such peptides. High levels of antioxidants in natural foods can be even more appealing than synthetic counterparts [24, 27]. In a study by Amarakoon [28] the amino acid profile of pea showed that pea grown in central Europe was rich in leucine, lysine and arginine which were sufficient for a normal diet. The amino acid profiles of pea were compared with soybean and reference FAO/WHO requirements. The essential AA content was higher in pea in comparison to soybean. The lysine content was 6.39–6.93/16gN in pea, which was also higher than soybean. Another comparison of AA profile of flour and isolates and concentrates of protein of pea, soybean and lupin was made by Tomoskozi et al. [29]. They concluded that composition of AA was the same in all compounds with the highest amount of glutamine and comparatively lower amounts of aspartic acid, lysine and arginine and smallest contributions of methionine, cysteine and tryptophan.

In comparison to soybean and lupin, pea compounds had high levels of arginine, methionine and valine and comparatively low levels of cysteine and glumatic acid.

Amino acids	cv. *ucero*	cv. *ramrod*	cv. *agra*	*cv. terno*	*cv. Xantos*	*cv.suit*	*cv. achat*
Non-essential amino acids							
Asp	10.39	10.08	9.98	10.87	10.55	10.69	10.58
Glu	17.09	16.49	15.43	15.07	16.19	15.96	16.16
Ser	4.89	4.80	4.77	4.23	4.16	4.05	4.25
Gly	8.16	8.26	7.85	4.11	4.0	3.98	3.92
Arg	5.76	4.93	4.12	9.36	8.60	9.68	8.32
Ala	5.17	6.35	5.75	4.19	3.88	3.83	3.79
Pro	3.62	3.64	3.52	3.77	3.57	3.64	3.63
Essential amino acids							
His	1.07	1.13	1.03	2.22	2.16	2.18	2.16
Val	3.85	3.89	3.61	4.72	4.29	4.34	4.32
Met	0.65	0.70	0.70	5.0	1.08	1.05	0.99
Cys	0.30	0.37	0.39	2.01	2.03	1.9	1.67
Ile	3.51	2.64	2.52	4.23	3.86	3.77	3.9
Leu	5.72	6.51	7.01	7.11	6.45	6.33	6.55
Phe	5.07	5.06	4.59	4.87	4.59	4.33	4.56
Tyr	3.98	3.76	3.77	2.79	3.18	2.87	3.18
Lys	18.34	19.69	17.03	6.93	6.55	6.39	6.63
Thr	3.04	4.22	6.92	3.45	3.64	3.34	3.53
Trp	0.02	0.02	0.02	n.a.	n.a.	n.a.	n.a.

Table 2.
Amino acid profile of different pea cultivars [7, 25].

The muscle development and growth in human body is dependent on postprandial essential amino acid availability particularly leucine [30]. AA composition, essential AA content and anti-nutritional factors regulate the availability of essential AA [31]. Thus, variation in AA composition particularly in essential AA are desirable for improving AA profile of pea proteins. Natural variation among varieties for AA profile is present as depicted in **Table 2**. Wide crosses and mutants can be searched for more desirable AA profile of pea proteins. Furthermore, introgression approach can be deployed for improvement of existing germplasm using a natural variation.

4. Seed storage proteins

Apart from protein comprising a major part of the seed, the other constituents include 1.5–2% fat, minerals, vitamins, polyphenols, oxalates, saponins and phytic acid [32–34]. Starch and dietary fibre account for 60 percent of carbohydrate content and rest include non-starch part of carbohydrates comprising sucrose, cellulose, and oligosaccharides (**Figure 1**) [34, 36]. Protein and the starch fraction of seed show high variations, whereas the other components remain comparatively constant [18]. Pea proteins are classified based on Osborne fractionation [37] into two different categories, i.e., globulins soluble in salt and albumins soluble in water which collectively account for 80% of the pea seed protein. Young embryos after germination of seed obtain nitrogen from globulins and some of

the albumins which are also known as storage proteins. Globulins are further divided into two categories based on coefficients of sedimentation, i.e., legumin (11S fraction), vicilin and convicilin (7S fraction) as shown in **Figure 2**. The two classes differ from each other in structure and molecular weight. Legumin has a molecular mass ranging from 300 to 400 kDa and hexameric protein form. There are three polypeptide families of legumin, and sequence similarities differentiate them into various groups. The LegA polypeptide comprises of legA, legB, legA2, legC, and legE, LegJ polypeptide comprises leg J, legK, legL and legM whereas LegS is single member of family [39, 40]. The LegA and LegJ families comprise an apparent subdivision with the molecular mass of 65 kDa, and on the other hand, the apparent subdivision of LegS has *) kDa molecular mass. Only a single peptide of legumin is imported to the endoplasmic reticulum and removed during translation. Ultimately, trimers of legumin peptide are formed and moved to the

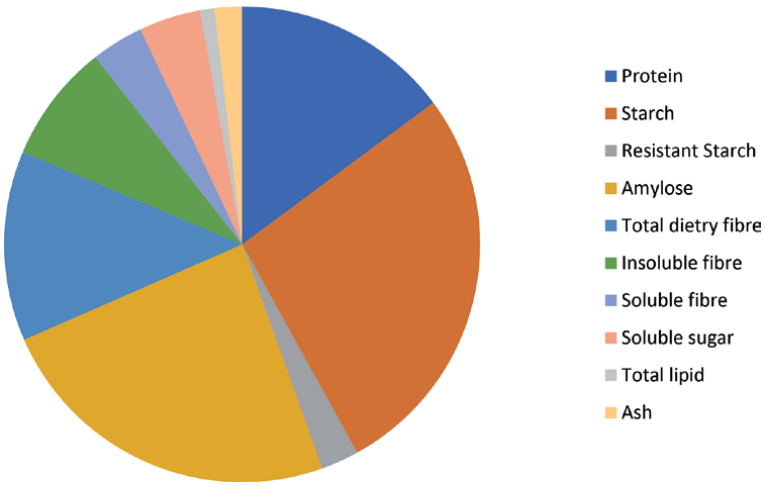

Figure 1.
The average composition of pea seeds [35].

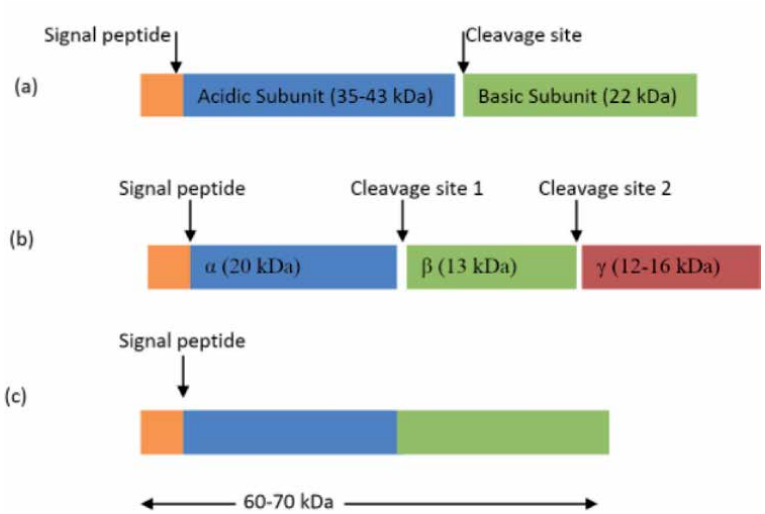

Figure 2.
Size of subunits of pea proteins, including the cleavage site of (a) Legumin (b) Vicilin (c) Convicilin [38].

pre-vacuolar compartment [41]. Furthermore, the peptides are processed into basic and acidic polypeptides of 20 and 40 kDa with the help of vacuole processing enzyme and the two peptides are linked by disulphide bridge. A complete protein structure is assembled from trimers to hexamers. The molecular weight of vicilin is 47–50 kDa and it forms trimers of 150 kDa molecular mass [42]. Only some vicilins undergo cleavage at post translational level [43]. Vicilin contains two cleavage regions which are separately processed. Three fragments of 13 kDa (â), 20 kDa (R) and 16 kDa (γ) are obtained by cleavage in both regions. Two fragments of 25 kDa (â + γ) and 20 kDa (R) are obtained, if site A is cleaved and two fragments of 16 kDa (γ) and 36 kDa (R + â) are obtained if site B is cleaved [43, 44]. Noncovalent bonds held processed peptides [40, 44]. Glycosylation takes place near to C terminus of γ subunit of vicilin polypepetides as they are glycosylated [45]. Trimers of 210 kDa molecular mass are formed by convicilin protein having a molecular mass of 70 kDa. Heteromeric trimers comprising convicilin and vicilin polypeptides also occur [2, 46]. Elimination of single peptide is only reported post translational modification in the case of convicilin and glycosylation is absent [47]. Convicilin and vicilin show sequence similarity of amino acids at C terminus whereas N terminal being highly charged have different sequences between two polpeptides [48, 49]. Based on isoform, sequence similarity occurs between 122 and 166 amino acid residues. Physiochemical properties of globulins are different, owing to variations in molecular weight and structure.

The water-soluble albumin proteins have 5–80 kDa molecular mass and consist of enzymes and anti-nutritional factors such as amylase inhibitors, lectins and protease inhibitors [32]. Further two classes are obtained in albumins, i.e., albumin protein with two polypeptides having 25 kDa molecular weight and another with 6 kDa molecular weight [46]. Minor portions include prolamins which are soluble in diluted alcohol and glutenins, which are soluble in diluted acid [32]. The protein structure can be altered by external factors such as temperature, pH and salts during the extraction process resulting in different surface features and conformations.

The globulin protein classes, i.e., vicilin and legumin in different concentrations, can make good gels, whereas convicilin is known to hinder gel formation [50]. The food industry needs raw material with desirable composition of globulin in peas like high levels of vicilin and legumin or low levels of convicilin [40]. Further, gel making property not only depends on the composition of globulins but also matter of isoforms of isolate [51, 52]. The genetic variation in the composition of globulins and decreased levels of anti-nutrients in albumin fraction of pea proteins are desirable material for development of new varieties using breeding techniques. Natural variation is reported in case of the protein content of pea and its composition, which can be used in breeding programs [53–55]. The r locus in the pea genome is known to control the starch synthesis, which shows pleiotropism with protein content and its composition [56, 57]. With the advancement of techniques for elucidating in planta processing of proteins, there will be more clues for the controlled composition of proteins using genome editing techniques.

5. Seed crude protein determination in pea

5.1 Protein isolate extraction methods

Alkaline extraction/isoelectric precipitation (AE/IEP) – This method utilizes the high solubility of pea proteins in alkaline conditions and their minimal solubility at isoelectric point (pI) at pH between 4 to 5 [32]. This method is the most common for legume protein extraction, and it takes advantage of similar solubility characters

for legumin and vicilin [33, 58]. The de-fatted flour of legume (with or without seed coat) is dispersed in water and pH is adjusted to an alkaline range using NaOH, KOH or $Ca(OH)_2$, and further left for 30–180 mins for maximizing protein solubility [32, 33]. Without de-fatting process, the protein-lipid interaction limits the solubility of protein leading to decrease in the isolated yield, and the temperature can be increased to 50–60°C to aid solubilization [59, 60]. The protein denaturation can be limited by avoiding the higher temperatures. The mixture is further centrifuged, and supernatant is collected, and isoelectric pH is adjusted using HCl or H_2SO_4. The precipitated protein is collected after centrifugation and washed, neutralized, and dried by drum or freeze drying [32, 33]. The isolate yield can be increased up to 80–94% by optimal processing conditions and the conditions used in a process can affect the purity, yield and functionality of the isolate [58]. Hoang [58] evaluated that the extraction pH and flour: water ratio were most critical factors. The flour: water ratios of 1:5 to 1:20 (w/v) was reported [32] but Hoang [58] stated that the increase in concentration gradient between the solid and liquid phase in low ratio slurry can increase solubility. Although high alkalinity increases the isolate solubility and yield of protein, but the pH 11 and above are basically associated with increase in swelling of starch, leading to contamination of starch in isolate product [58]. Alkaline Extraction is also responsible for the adverse chemical reactions like the conversion of serine and cysteine residues to lysinoalanine compounds (nephrotoxic), decreased proteins bioavailability, and racemization of amino acids [61, 62]. The processes employing high alkaline pH, high temperature is associated with high yield of isolate, but there is high susceptibility of denaturation of isolate [61, 63]. The particle size of flour and solubilizing agent used can also affect the yield of isolate. The optimum particle size for flour is 100–150 μm and it was reported that NaOH and KOH generate more yield in comparison to $Ca(OH)_2$ [64]. Also, there was protein loss of 6.2% from discarded supernatant from this extraction method [58] and in place of IEP, ultrafiltration (UF) or diafiltration membranes with specific molecular weight cutoffs can be utilized for isolating proteins of interest from the supernatant [32]. The efficiency of extraction can be improved by alteration in the molecular weight cutoffs, membrane type, concentration, and volume of the filtrate and addition of diafiltration to UF techniques [65]. The albumin proteins can be recovered by controlling these factors and further result in enhancing yield of isolate and alteration in isolate functionality leading to reduction in effluent losses. The use of UF can provide milder conditions for extracted proteins, so that their functionality can be enhanced and it gives higher yields in comparison to IEP [66].

Boye et al. [65] also confirmed that there were slightly higher protein levels in UF than the IEP process. Membrane filtration is also effective in reduction of anti-nutritional compounds in isolate [65]. Taherian et al. [67] conducted a study for functional properties of commercial and membrane-processed yellow pea protein isolates. The use of UF results in reduction of phytic acid upto 28–68% and possess improved functionality (e.g., solubility, rheology, foaming and emulsification) for commercially available isolates. The solubility of the commercial protein isolates was reported as ~20% vs. ~80% by using UF/diafiltration at pH 2.0. Fuhrmeister and Meuser [68] found the enhanced solubility, emulsifying, foaming and fat-holding properties by UF recovery of proteins from wrinkled pea relative to heat, acid, and heat/acid precipitation.

5.2 Salt extraction (SE) and micellization

SE has advantage of the salting-in and out phenomenon of proteins which is followed by desalting for lowering the ionic strength of protein environment [32, 69]. In this process, the flour is stirred in salt solution of ionic strength

(1:10 (w/v) ratio) for 10–60 mins and further followed by removal of insoluble matter by settling, screening, decanting, filtering or centrifugation. The supernatant is desalted and dried [32, 69, 70]. The choice or concentration of salts is selected according to salting-in and salting-out characteristics of the protein and any unwanted proteins, respectively because the proteins precipitate at an array of ionic strengths [71, 72]. The salting-in of proteins generally occurs at ionic strength (between 0.1 to 1 M) [60] and the other factors include interactions of salt and sample components and ensuring the use of food-grade salts [69, 73]. The major advantage for this technique is that extreme level of acidic or alkaline pH alongwith elevated temperature is not required. The extraction occurs at pH level of 5.5–6.5, but Crevieu et al. [74] reported slightly alkaline pH for increasing protein solubility [69]. The pH can be maintained by the addition of acid or base or a salt solution with buffering capacity can be used. The supernatant with extract of high-salt protein should have a protein concentration of 15 to 100 mg/mL [69] and many methods have been used for decreasing its ionic strength.

In the process of micellization, protein precipitation is induced by adding cold water at a ratio of 1:3 to 1:10 (v/v) of high-salt protein extract to water [69, 75]. The solubilized proteins can be adjusted to low ionic strength by the dilution of protein solution through different dissociation reactions which forms loosely associated and low molecular weight aggregates. After reaching a specific concentration of protein, the aggregates can re-associate into low molecular weight species, known as micelles [69]. The arrangement of micelles is as thermodynamical spheres with minimum interfacial energy by giving exposure to polar moieties in outer aqueous environment and hydrophobic moieties towards the center. The proteins possesing more surface hydrophobicity have more protein–protein interactions and are also more successful for creating large and uniform aggregates [69]. The diluted solution can be left to stand for certain time for increasing micelle formation. This is followed by centrifugation and further the pellet is dried, and the high salt aqueous solution is discarded [32, 69]. Mwasaru et al. [75] reported that after using 0.25 M NaCl solution at pH value of 6.5 and 6 hours of micellization standing time, the protein extractability for pigeon pea and cowpea was yielded a 40.2% and 36.7%, respectively and these values were further compared to alkaline-extracted samples at pH value of 10.5 and 8.5, respectively, where the yields increased with respect to alkalinity. Gueguen [36] evaluated that 95% yield can be attained using micellization method.

5.3 Dialysis

The another commonly used method for desalting is dialysis. It is the process of membrane separation driven by a potential gradient for diffusing water and other solutes with low molecular weight like, salt and this process carried out using semipermeable membrane [72]. Gueguen et al. [70] and Crevieu et al. [74] used pea protein membranes with cutoffs of 8000 Da and 12,000–14,000 Da, respectively. The diffusion requires time for causing equilibrium on both sides and is complete when the potential gradient becomes negligible [72]. The changes in fresh, precooled liquid against which the sample is dialyzed helps in ensuring that very low concentrations of solutes remain in the sample. Gueguen et al. [70] cited a process of 130 hours which requires five changes of water of 20 times the extract volume. Crevieu et al. [74] dialyzed solution of globulin against two changes of 10 times the extract volume of ammonium carbonate, that requires 70 hours and results in a yield of 66.8%. Dialysis can also be used for separation of gloulin and fractions. According to the protein classification of Osborne, the dialyzed sample is centrifugated and it results in dissolved albumin fractions in supernatant and precipitated fractions of globulin in the pellet [70]. The phenolic compounds present in pea can

be reduced by additional steps during processing, like the use of alcohol washes and charcoal filters. The cross linkage of proteins can be improved by antioxidant activity of phenolic compounds which can negatively affect protein digestibility and enzymatic activity, leading to undesirable color and flavor compounds within the food product.

6. Food applications of pea proteins

The application of bioactive ingredients (hydrophobic, hydrophilic compounds, minerals, and probiotics) is less due to their instability, less bioavailability, and unsuitable flavors in the food system. So, encapsulation can be a promising technique for solving these problems related to bioactive ingredients. Nowadays, there is an increase in research for pea protein as encapsulating materials, because of its health benefits, nil genetic modifications, and hypoallergenic issues [76]. As many researchers have recognized the importance of natural polymers for preparing biodegradable packaging and since pea protein acts as a biodegradable and biocompatible natural polymer, it can be used for producing biodegradable films. It can provide promising possibility for the application of pea proteins for making biodegradable films in industrial-scale food production.

There are extrusion techniques which include low-moisture extrusion (LME, 40%) and high-moisture extrusion (HME, >40%), these techniques are widely used in commercial food production. LME is generally used for preparation of snacks and HME is used basically for meat analogue preparation. The research of pea protein based extruded products is very common nowadays and many researchers reported that pea protein was used in different starches like rice starch [77–79] wheat starch [80] and corn grits [81] for preparing protein-fortified extruded snacks by LME, and the results concluded that pea protein-fortified extruded products exhibits high content of protein and possess balanced amino acid profile in comparison to pure extrudates of starch.

There are many studies which report that by the addition of pea protein in cereal products can improve the nutritional value of the product because pea protein provides the essential amino acids and improve the texture of cereal product [4, 82–85]. The plant protein can be used as substitute for animal protein for meeting nutritional need of lacto-vegetarians and thus can make the food healthier. Several researchers are working on partly or fully substitution of dairy proteins with pea protein and the impact on taste and structure of these products [86–90].

7. Conclusion and future prospects

Based on the literature reviewed in this chapter, we think that analogous research and advancement on pea proteins would be required if any significant boost in pea protein utilization is envisaged. While pea protein isolates have usually been discussed in the research literature as relatively mundane, you will find very few sensory analysis information to help the claim. The main limitation on the sales of pea protein meals components is the trouble in fighting with the well-established, versatile soy protein items which dominate the meals protein market. Soy proteins are already available for a very long time, and research by the main producing businesses has resulted in several tailored items for programs. Pea concentrates and flours are generally referred to as having a terrible taste (beany, bitter). The incorporation of pea concentrates and flours into meals products such as bread, is usually restricted by flavour problems. This truth is insignificant within the

foods ingredient industry because proteins in this particular marketplace are sold primarily by functional qualities and price. Although to be used in food aid plans for developing nations, this's of concern and demands that pea protein is together with a protein source that will offer a comprehensive source of sulfur amino acids. In pet feeding, the nutritional value of protein sources is likewise essential. Feeding studies show that pea protein requires supplementation with methionine to get it with the nutritional value of soy protein.

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Chapter 8

Functional Uses of Peanut (*Arachis hypogaea* L.) Seed Storage Proteins

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Abstract

Peanut (*Arachis hypogaea* L.) is an important grain legume crop of tropics and subtropics. It is increasingly being accepted as a functional food and protein extender in developing countries. The seed contains 36% to 54% oil, 16% to 36% protein, and 10% to 20% carbohydrates with high amounts of P, Mg, Ca, riboflavin, niacin, folic acid, vitamin E, resveratrol and amino acids. Seed contains 32 different proteins comprised of albumins and globulins. The two-globulin fractions, arachin and non-arachin, comprise approximately 87% of the peanut seed proteins. Peanut worldwide is mainly used for oil production, consumption as raw, roasted, baked products, peanut butter, peanut flour, extender in meat product formulations, confectionary and soups. Peanut proteins have many properties such as good solubility, foaming, water/oil binding, emulsification that make them useful in various food products. Very limited studies have been carried out in peanut functional properties, which has been reviewed in the present article. Adequate modifications can be done in protein functionality that are influenced by pH, temperature, pressure etc. However, some individuals develop severe IgE-mediated allergies to peanut seed proteins. Thus, methods to improve nutrition and reduce allergenicity have also been discussed. Within the last decade, manipulations have been done to alter peanut chemistry and improve nutritional quality of peanuts and peanut products. Hence, improved comprehensive understanding of functional properties and nutritional chemistry of peanut proteins can generate better source of food grain to meet nutritional requirement of growing population. In the present review, composition of peanut seed proteins, functional properties, nutritional components and nutraceutical value have been discussed with respect to beneficial aspects to health, reducing hunger and usage in food end products.

Keywords: seed proteins, albumin, globulin, nutritional value, functional properties

1. Introduction

Peanut, or groundnut, of genus *Arachis* is a member of the legume family (Fabaceae). Cultivated peanut (*Arachis hypogaea*), an allopolyploid with AABB genome composition, is the second-most important grain legume crop worldwide after soybean [1]. The genus *Arachis* is endemic to South America [2] and the probable center of origin of *Arachis hypogaea* has been recognized in Gran Panatanal (Mato Grosso, Brazil) and also on the eastern slopes of the Bovilian Andes [3].

Peanut has now become one of the major global oil-seed crops covering approximately 26 million ha land area in about 120 countries [4–6]. According to FAO, world production of groundnut is above 45 million tons, averaging about 1.8 t/ha. Significant level of annual peanut production has been recorded in India amounting to approximately seven million tons [7]. China leads in production of peanuts, with a share of about 41% of overall world production, whereas India has 14% share and the United States has (7%) [8]. *Arachis hypogaea* has been divided into two subspecies *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata* [9]. Morphological variations in branching and flowering patterns, pod and seed traits are used to characterize different botanical varieties [10]. The varieties are further distinguishable into a number of market types or cultivars like Virginia (large seeded), Runner (small seeded), Peruvian runner, Valencia and Spanish type. Some market types or cultivars are more preferred for some particular uses due to differences in flavor, oil content, size, shape, and disease resistance [11].

Peanut is accepted as a potential source of food grade protein and an energy dense food. The seed typically contains 36% to 54% oil, 16% to 36% protein, and 10% to 20% carbohydrates as well as high amounts of macro minerals, trace elements, vitamins riboflavin, niacin, folic acid, fiber and vitamin E, resveratrol, phytosterols. It is also known as poor man's nut and being seen as potential functional food. A 100 g of peanut kernels provide 567 kcal of energy and 8.5 g of dietary fiber [12]. Consumption of peanuts can reduce risk of inflammation and diseases like diabetes, cancer, gallstone and alzheimer's [12, 13].

Peanuts are consumed all over the world in a wide variety of forms such as raw, boiled or roasted, and are widely used to prepare a variety of packaged foods (peanut butter, candies, confections, and snack products) in the United States. Peanuts and peanut butter contain monounsaturated fatty acids besides plant proteins, minerals like magnesium, potassium, fiber, arginine and various bioactive components. The by-product of the oil extraction is a meal that is also high in proteins, dietary fiber, antioxidants, vitamins, and minerals, and can be utilized as animal feed or processed further for human consumption. The defatted protein flour after oil extraction in peanuts, has immense uses and has been exploited in meat-like products that can be used to formulate cholesterol-free vegetarian alternatives [14]. Peanut flour is used in composite flours with non-wheat cereals to improve the nutritional value of bread [12]. Peanut bars, peanut milk and fermented peanut are also different forms of consumption. Protein energy malnutrition in third world developing countries is a problem due to dependence on animal proteins which are expensive and thus, affordable plant proteins with its additional benefits are being exploited such as peanut proteins, which can be used to combat protein-energy malnutrition. Partially defatted peanut flour, is a protein-rich, inexpensive and underutilized product that offers the same health and dietary benefits of peanut with less fat and can be utilized for making value added products to eradicate malnutrition among children [15]. Peanut proteins play an important role in many food products because of their properties such as nutritional value, contribution to food texture, solubility etc., among others. Huge tons of by-products obtained from peanut industries can be utilized to generate a reasonably high quantity of protein, that could be further used in a variety of food formulations due to its properties such as water and oil absorption, gel formation, foaming, emulsification etc. Functional properties depend upon extraction procedure and use of adequate modification methods [16].

Thus, the present chapter focuses on the peanut seed storage proteins composition, nutritional value, bioactive components, functional properties, its usage and methods to reduce allergenicity.

2. Peanut proteins

Seed storage proteins are present as one or more groups of proteins in high amounts in seeds to provide a store of amino acids for use during germination and seed growth. The peanut seed contains 32 different proteins comprised of albumins and globulins. The seed storage proteins are mainly composed of arachin (legumin), conarachin (vicilin) - I, II fractions [17]. Many papers have highlighted the composition of seed storage proteins (SSPs) using one dimensional and two dimensional PAGE [18–21]. The different fractions of seed proteins by SDS PAGE in groundnut cultivars is shown in **Figure 1**. SSPs in peanut are composed of families of 2S, 7S, and 11S proteins that can be subdivided in homology groups. 11S proteins are more diverse than 2S and 7S proteins in peanut seeds, but 2S and 11S subgroups are very similar in the A and B genomes of *Arachis* [20].

However, peanut proteins is also a source of severe IgE-mediated allergies in some individuals. Due to peanut being recognized as one of the potent allergens, the immunological protein names with prefix *Ara* with different numbers have also been designated from Ara h 1 to Ara h 17.

2.1 Globulins

Globulins (7S and 11S) comprise of the majority of the total protein in many seeds that are consumed by humans. Vicilins (7S globulins) and legumins (11S globulins) share similar folds and belong to the cupin superfamily of proteins.

Ara h 1, a 7S vicilin, exists as trimer formed by three identical monomers and is also a glycoprotein. It compromises approximately 12–16% of peanut proteins [22]. When analyzed in SDS PAGE, the Ara h 1 vicilin shows two isoforms at 69 and 66 kDa [23]. 11S globulin seed protein is a hexamer (360–380 kDa) formed by two trimers [24], with each monomer having four linear epitopes [25]. Ara h 3 is a legumin-like seed storage protein that has high sequence similarity to glycinin, the major 11S globulin seed storage protein family in soybean. Ara h 3 and related proteins belong to the 11S globulin storage protein family that is characterized by three common features. The first one is that they contain an acidic and basic chain separated

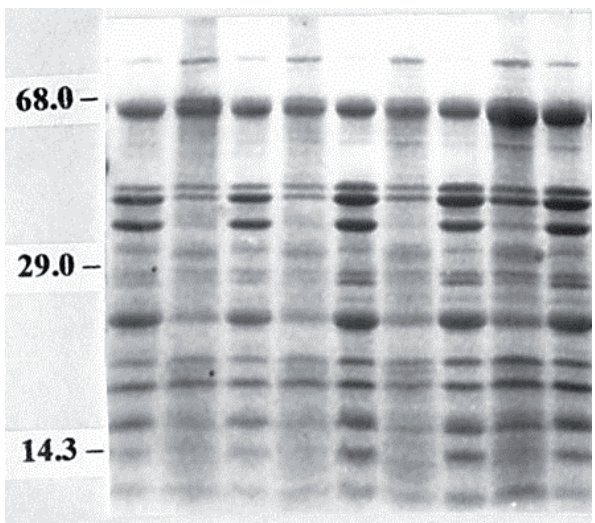

Figure 1.
SDS PAGE analysis of Total Seed Storage Proteins in Peanut (Arachis hypogaea) cultivars along side a marker (adapted from [19]).

by a conserved Asn-Gly (N–G) peptide bond. Second, the formation of intra and inter-disulfide bonds is observed due to four conserved cysteine residue. Third, an Asn-Gln (N–Q) peptide bond is present that serves as a potential proteolytic cleavage site. It also functions as a trypsin inhibitor [24, 26, 27]. Ara h 3 and soybean glycinin result in a sequence identity of 47.2%. Mature Ara h 3 is a hexamer (360–380 kDa) formed by a head-to-head association of two trimers [25, 28]. Ara h 3 was originally identified as 14 kDa [28]. Later, it was found to be of 60 kDa [24]. This Ara h 3 is post translationally modified and cleaved into 43 kDa acidic and 28 kDa basic subunits. In SDS PAGE, several fragments can be identified as 14, 25, 42 and 45 kDa.

Ara h 4 is also arachin, an isoform of Ara h 3. Ara h 4 is no more used but it is renamed as Ara h 3.02 [29]. Five different genes were found to encode for isoforms of Ara h 3 [30].

2.2 Albumins

Ara h 2, a 2S albumin is a glycoprotein and accounts for approximately 6 to 9% of total peanut protein [31] with a molecular weight of approximately 17 kDa [32]. Ara h 2 also known as conglutin and functions as a trypsin inhibitor. Structurally, Ara h 2 has five α-helices arranged in right-handed super helix connected by several extended loops with four conserved disulfide bridges and 10 highly exposed epitope binding sites [33].

Ara h 5 (15 kDa) belongs to the profilin family and regulates the polymerization of actin [34]. It is presented at low levels in peanut extracts. Ara h 6 is a 15 kDa protein and belongs to the conglutin family [35]. It is 59% homologous to Ara h 2 and has similar allergenicity [36, 37]. Ara h 6 is a heat and digestion stable protein and showed resistance to proteolytic treatment [38, 39]. Ara h 7 is also a 15 kDa protein and belongs to the conglutin family [35]. The sequence identity between Ara h 2 and Ara h 6 is 35%.

2.3 Other proteins

Ara h 8 (17 kDa) is a Pathogenesis related protein. It is homologous to Betv1 proteins. Ara h 9 and Ara h 17 (9.8 kDa, 2 isoforms) are nonspecific lipid-transfer (nsLTPs) proteins of type 1 category. Ara h 16 is an nsLTP of type 2 category (approx. 7 kDa) [40].

Ara h 10 (16 kDa, 2 isoforms) and Ara h 11 (14 kDa) belong to oleosin. Ara h 14, Ara h 15 are peanut oleosins with amphiphilic structural proteins. Ara h 12 and Ara h 13 are defensin, with molecular weight ranging from 5 to 12 kDa [35]. Oleosins are also abundant in peanut seeds.

3. Importance as a functional food

Peanut is increasingly recognized as a functional food. The protein quality is based on amino acid pattern and percent digestibility. According to Protein Digestibility Corrected Amino Acid Score (PDCAAS), the plant protein peanut is nutritionally equivalent to animal proteins such as meat and eggs. The PDCAAS for peanuts has been estimated to be about 0.70 out of 1, whereas, for whole wheat PDCASS is 0.46. After the oil is extracted, the protein content in peanut cake can reach upto 50% [41]. All the protein components are highly digestible.

3.1 Amino acids content

Peanuts contain all the twenty amino acids, including 9 essential amino acids, necessary for normal body growth and metabolism [12]. They also show high levels of arginine and histidine. The remaining amino acids were present in substantial quantities except methionine, tryptophan and cystine that were considered low [42].

Comparisons across common tree nuts and peanuts show that all are naturally high in both acidic and basic amino acids, in addition to also being naturally high in hydrophobic amino acids, including leucine, glycine and valine, among others [43]. Peanut has a high percentage of arginine (12.5%), which gives added benefit with its overall high protein content, making peanut an important dietary source of this amino acid whose consumption has been directly linked to various cardiovascular health promoting activities [14]. The arginine is highest in peanuts among foods [44]. The amino acid profile of the peanut meals shows that it can be an ingredient for protein fortification [45]. Being a leguminous plant protein, it also has additional components that have positive health benefits like fiber and unique bioactive components besides amino acids (**Tables 1** and **2**). They also contain many important functional components including coenzyme Q10 [46], and polysterols, which make it a functional food [47, 48].

Amino acid data for blanched seed (without peanut skin or testa) is ultimately most relevant to peanut nutrition as the skin only accounts for approximately 3% of the total seed weight after shelling and skins are relatively low in total protein compared with the blanched seed, i.e., approximately 15% versus 25%. For blanched seed, asparagine/aspartic acid and glutamine/glutamic acid residues predominate, accounting for approximately 35% of the amino acids, which is in good agreement with data from other sources [43, 49].

3.2 Fats, vitamins and minerals

In the fats, peanut contains 50% monounsaturated fatty acids (MUFAs), 33% polyunsaturated fatty acids (PUFAs) and 4% saturated fatty acids [50]. PUFA needs to be given through diet and cannot be synthesized. This way presence of high MUFAs and PUFAs reduce heart stress. So, the oils of peanuts are very important and healthy.

Carbohydrates that contain fiber or starch, these two types of carbohydrates have a slower, less pronounced effect on blood sugar. The American Diabetes Association ranks peanuts and other nuts as diabetes superfoods. Peanut have a low glycemic index (GI) and glycemic load (GL) [51]. On a 100 –point scale, the GI of peanuts is 14, and the GL of peanuts is one. Mature groundnut kernels were reported to contain 9.5–19% carbohydrates in which starch and sucrose are the major constituents.

The peanut is also a good source of minerals like Magnesium, Calcium, Phosphorus, potassium, iron, zinc, iron, copper, selenium and vitamins as well as dietary fibers (**Table 1**). Minerals like calcium and phosphorus are important for normal growth and development of bones and muscles. While minerals required in trace amounts like zinc, selenium whose daily requirement can be met by 100 g of peanuts [12, 52].

Peanuts are a vital source for introducing most of the water soluble vitamins into the human body along with vitamin E which is fat soluble [1, 12]. A 100 g peanuts consumption is capable of providing up to 75% recommended dietary allowances (RDA) of Niacin, 60% RDA of folate, 53% RDA of thiamin, 10% RDA of Riboflavin, 35% RDA of pantothenic acid, 27% RDA of pyridoxine, 55.5% RDA of vitamin E [12, 52]. In 42 g of peanuts, more than 10% provide recommended dietary allowances (RDA) for niacin, pantothenic acid, and total folate is present.

Another important vitamin which is supplemented in the body by the intake of peanuts is vitamin B3 [53] (known as Niacin or Niacinamide or Nicotinamide), to an extent of 13.525 mg. The vitamin B5 pantothenic acid is also provided by peanuts [52]. This vitamin plays an important role in the normal functioning of the respiratory chain and participates in hydrogen transfer, and electron transfer reactions through its coenzymes, Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP). Roasted peanuts will provide B6 to the human body to the extent of 0.256 mg. Vitamin B9, more commonly known as folate or folic acid, is a water-soluble vitamin that is part of the B vitamin family and required for normal

functioning. Folate (vitamin B9) present in peanuts to an extent of 145 μg may also help protect against cancers of the lung, colon, and cervix [12].

Peanut flour which is most commonly used for fortification contains protein ranging in between 47% - 55% i.e. a good amount of protein. Peanut flour has been

COMPONENTS	CLASS	TYPES	AMOUNT (per 100gm of dry roasted peanuts)
Lipids	Fatty acids	Saturated	6.893 gm
		Monosaturated	24.640 gm
		Polysaturated	15.694 gm
Vitamins	Fat soluble	E (tocopherol)	8.2 mg (raw), 4.1 mg/ 100 g roasted
	Water soluble	B2 (Riboflavin)	0.098 mg
		B1 (Thiamine)	1.0 mg
		B5 (Panthothenic	1.395 mg
		acid)	13.525 mg/
		B3 (Niacin)	0.256 mg
		B6 (Pyridoxine)	145 mg
		B9 (Folate)	55.3 mg
		Choline	
Minerals	Macro	Potassium	658 mg
		Sodium	Approx.5.56 mg
		Calcium	54 mg
		Magnesium	175 mg
		Phosphorus	358 mg
	Micro	*Selenium	7.5 mg
		*Copper	0.671 mg
		*Manganese	Approx.2.06 mg
		Iron	2.26 mg
		Zinc	3.31 mg
		(* antioxidant minerals)	
Amino acids	Essential	Tryptophan	0.230 gm
		Leucine	1.535 gm
		Isoleucine	0.833 gm
		Methioione	0.291 gm
		Phenyalanine	0.304 gm
		Valine	0.993 gm
		Lysine	0.850 gm
		Threonine	0.811 gm
	Non- essential	Glycine	1.427 gm
		Alanine	0.941 gm
		Cysteine	0.304 gm
		Tyrosine	0.963 gm
		Arginine	2.832 gm
		Histidine	0.599 gm
		Aspartic acid	2.888 gm
		Glutamic acid	4.949 gm
		Proline	1.045 gm
		Serine	1.167 gm
Others	Total carbohydrates		21.51 gm
	Dietary fibers		8.0 gm
	Functional components	Coenzyme Q10	
	Total Sugars		4.18 gm

(Adapted from Source USDA 2011)

Table 1.
Nutrient components in Peanut.

BIOACTIVE COMPOUNDS	TYPE	AMOUNT (per 100 gm of dry roasted peanuts)
Isoflavonoid	Daidzein Genistein	49.7 mg 82.6 mg
Phenolic acids	p-coumaric acid	6.9 mg
Phytosterols	b-sitosterol	61 mg to 114 mg
Stilbenes	Resveratrol	0.48 mg to 3.96 mg

Table 2.
Composition of bioactive compounds.

used to replace animal proteins in a variety of products. Peanut flour blends well with cereal flour to yield products with excellent flavor texture and color [12].

4. Presence of secondary metabolites

Peanut is a reservoir of secondary metabolites like flavonoids, polyphenols, phytosterols, stilbenes (**Table 2**). The evaluation of peanuts role in a heart-healthy diet has increased in the last decade [54]. Extraction procedures would play a big role in getting these bioactive components since extracting solvent, isolation procedures, purity of active compounds, as well as the test system and substrate to be protected by the antioxidant affects its function [55].

The flavonoid content in peanuts was determined, which is second only to walnuts [56]. Studies [57, 58] reported that peanut seeds had an isoflavonoid content of daidzein and genistein in the greatest amounts with a content of 49.7 mg/100 g and 82.6 mg/100 g, respectively. A-type proanthocyanidins was determined in peanuts [59]. Luteolin was the principal antioxidative component from the methanolic extracts of peanut hulls [60]. Mature, red peanut skins contain about 17% by weight of procyanidins, nearly 50% of which are low molecular weight oligomers [61]. Catechins, A-type and B-type procyanidins dimers, trimers, and tetramers were also detected in chemically purified peanut skin aqueous and ethanol extracts [45]. Furthermore, higher concentrations of compounds mentioned were observed in raw peanut skins than roasted peanut skins.

The polyphenolic content of raw and dry roasted peanut samples containing varying levels of oleic acid (normal, mid, and high) were determined [62, 63]. Normal oleic acid peanuts had higher concentrations of individual polyphenolics than mid- and high-oleic peanuts. Free p-coumaric acid, three esterified derivatives of p-coumaric, and two esterified derivatives of hydrobenzoic acid were identified as the predominant polyphenolics. Whole raw peanuts had a mean of 25 mg/kg of p-coumaric acid (from a range of 8 to 66 mg/kg among cultivars) and the value increased to an average of 69 mg/kg when peanuts were roasted at 175 °C for 10 min.

Peanuts as a source of phytosterol has been getting a lot of attention with new research findings identifying phytosterols like beta-sitosterol, sitosterol in peanuts and peanut products as cancer growth inhibitors, as well as protectors against heart diseases [64]. The phytosterol contents of peanuts and peanut products were analyzed. Results show that among the four cultivars studied, the Valencia peanuts in raw, dry roasted, and oil roasted, contained the highest phytosterol concentration [65]. Studies with sitosterol or mixtures of plant sterols have shown that they reduce serum cholesterol levels in humans by approximately 10%. This discovery has resulted in subsequent research to evaluate the effects of sitosterol derivatives on cholesterol absorption and serum cholesterol levels [48].

Stilbenes contain two phenyl compounds connected by a 2- carbon methylene bridge. They occur in nature in a rather restricted distribution. Stilbenes like isoflavonoids, are also classified as phytoestrogens. Most stilbenes in plants act as antifungal phytoalexins, compounds that are usually synthesized only in response to infection or injury. The most studied one is resveratrol. Resveratrol is one of the major stilbene phytoalexin compounds produced by grape berries and peanuts in response to stress like fungal infection, the presence of heavy metal ions, or ultraviolet (UV) irradiation [66]. Resveratrol was found to be present in substantial amounts in the leaves, roots, and shells of peanuts, but very little was found in developing seeds and seed coats of field-grown peanuts [67]. The phytoalexin content of peanuts, however, increases during germination and is enhanced by microbial infection, postharvest induction procedures such as soaking and drying; wounding (slicing and incubation); UV light exposure, among others. Raw peanuts soaked in water for about 20 hours and dried for 66 hours increased the resveratrol content between 45 and 65 times after the soaking treatment [66]. Boiled peanuts contain more resveratrol than peanut butter and roasted peanuts. Resveratrol has been associated with reduced CVD and reduced cancer risk. Resveratrol has been shown from in vitro, ex vivo, and animal studies to have many attributes that may provide protection from atherosclerosis, antiproliferative, and proapoptotic properties against breast, colon, prostatic, and leukemia cells [68].

5. Industrial properties and applications

Functional properties affect the behavior of proteins during processing, storage and in preparation of food and food components (**Table 3**). Among different proteins, glycinin is nutritionally superior to the 7S con-glycinins [69], and possesses superior intrinsic functional properties for processed foods [70]. Processing technology has the capability of altering the protein structure, function, and physicochemical properties of peanuts [71–76].

5.1 Protein solubility, emulsification

Protein solubility is the first and foremost property that is determined in testing a new protein isolate. The functional properties of proteins are often affected by

Functional Property	Food type
Solubility	Beverages
Water absorption and binding	Meats, Sausages, breads, cakes
Viscosity	Soups, gravies
Gelation	Meats, curds, cheese
Cohesion –adhesion	Meats, Sausages, baked goods, cheeses, pasta products
Elasticity	Meats, bakery products
Emulsification	Sausages, bologna, Soup, cakes
Fat absorption	Meats, sausages
Flavor binding	Simulated meats, bakery goods
Foaming	Whipped toppings, chiffon deserts, angel food cakes

Table 3.
Uses of protein functional properties in food types (adapted from [70]).

protein solubility and those most affected are foaming, emulsification and gelation. The solubility of a protein is the thermodynamic manifestation of the equilibrium between protein–protein and protein solvent interactions [77]. These properties are affected by the intrinsic factors of protein such as molecular structure and size, and many other factors including the method of protein separation, production, pH, ionic strength and the presence of other components in the food system. The importance of these properties varies with the type of food products in which the protein concentrate is used.

Interactions of water and oil with proteins are very important in food systems because of their influence on the flavor and texture of foods. Proteins with high oil and water binding are desirable for use in meats, sausages, breads, and cakes [78]. Emulsification of proteins is closely related to the conformation of proteins and interaction of adsorbed molecules at the oil/water interface. Proteins with high emulsifying and foaming capacity are good for salad dressing, sausages, bologna, soups, confectionery, frozen desserts, and cakes [78]. Researchers [79–81] have determined the functional properties of several plant proteins concentrates using alkali solutions with isoelectric precipitation produced from peas and beans. The functional properties of peanut proteins have been subjects of limited studies that focused mainly on peanut flour [82–84]. According to some, protein isolates (PPI) have higher purity of proteins and better functional properties than other peanut protein products, such as flour or concentrate [82].

5.2 Influence of extraction procedure, pH, temperature

Functional properties of protein are influenced by many factors. For the end product uses, pH, temperature and ionic strength of the food system are important factors to consider. For initial extraction of proteins, methods and conditions of protein extraction, as well as downstream processing of extracted proteins such as purification, drying are the factors that are important [82]. Methods used to develop plant protein isolate/concentrate include isoelectric precipitation, alcohol precipitation, alkali solution and hot water extraction [83].

Peanut protein concentrates were isolated from defatted peanut flour by various methods such as isoelectric precipitation, alcohol precipitation, combined isoelectric and alcohol precipitation, and combined alkali solution with isoelectric precipitation [80]. Their functional properties (protein solubility, water holding/oil binding capacity, emulsifying capacity and stability, foaming capacity and rheology) were evaluated. Protein prepared by alcohol precipitation was found to have better functional properties particularly water holding/oil binding capacity, which were significantly different from other protein products such as of isoelectric and alkali precipitates [80]. The study concluded that it could be effectively used for making protein concentrates and suitable for use in various food formulations such as weaning foods, dry mixes, baked foods, whipped toppings and salad dressings owing to its high water and oil binding capacities.

Heating destroys anti-metabolites such as trypsin inhibitor in beans and nuts [85] and amylase inhibitors in legumes, thus improving the bioavailability or digestibility of the protein [86]. Roasting of peanuts significantly decreased protein solubility in peanut flour in the pH range 3.5–10.0 compared to that in raw peanut flour. Heating of peanut in water at 100–120 °C for 15 min decreased the protein solubility [16]. This might be due to the increase of surface hydrophobicity of protein via unfolding of molecules upon heat. The pH range also had a significant effect on the solubility of peanut protein [16]. The minimum protein solubility was observed at pH 3.5–4.5 and maximum solubility at pH 10 or higher 16]. The study suggested that solubility was pH dependent with the lowest solubility being

observed for both raw and roasted peanuts, at the isoelectric point of pH around 4.0. Protein solubility reduced as pH increased until reached an isoelectric point. At pH above the isoelectric point an increase in protein solubility was observed.

5.3 Gel forming ability or foaming

The abilities of protein to form gels and to provide a structure for holding water, flavors, sugars, and food ingredients are useful in food applications, and in new-product development that provide an added dimension of protein functionality. Foams are 2 phase systems composed of air bubbles surrounded by a continuous liquid lamellar phase [87]. Defatted peanut flour is not a good foaming agent, with a foaming capacity of only 6 ml/100 ml liquid, whereas, roasted peanuts showed half of the raw peanuts foaming capacity. Therefore, defatted peanut protein isolates may not be suitable in the food system that requires foaming such as cake and ice cream. Overall, roasting decreased functionality of peanut protein isolates, while fermentation significantly increased all functional properties of both raw and roasted peanut flours [16].

Recent studies have shown that high pressure treatment can change not only the functional characteristics of food proteins, but also their physical and chemical properties as well as molecular conformation [88–90]. In peanut protein isolates, high pressure treatment from 50–200 MPa, a non-thermal processing, significantly improved water binding capacity (WBC) and oil binding capacity (OBC). Additionally, pressure treatment could result in intensity denaturation of conarachin II fraction [91]. It was evident by SDS PAGE (**Figure 2**). This way protein isolates can be used as food supplementary material with improved characteristics.

Effect of membrane processing were analyzed on the functional properties, structural changes, subunit profile and sensory attributes of the groundnut protein concentrate [92]. Results indicated an increase in the nitrogen solubility and foaming capacity of the protein concentrate over pH ranges of 2–10, acid precipitated protein isolate. Protein concentrate also showed higher emulsion stability index, less hydrophobicity but reduced nutty flavor as compared to control flour and acid protein isolate. Thus, membrane technology could give a protein concentrate with improved functionality and sensory characteristics similar to roasted wheat and improved digestibility, which will have potential application in the development of

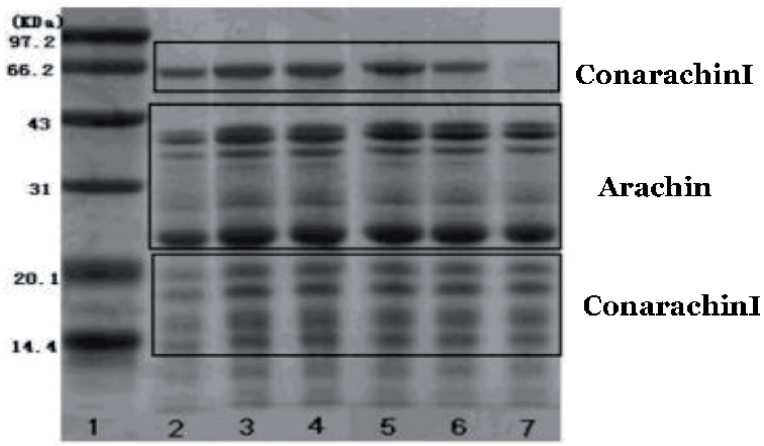

Figure 2.
Effect of high pressure on protein concentrates lanes 2(untreated) and 3–7(treated with 50-100 MPa). (adapted from [91]).

food product formulations. Increased thermal stability, protein solubility at 4.5–6 pH, improved foaming and emulsifying properties were noticed by conjugating protein isolates (PPI) with dextran [84].

5.4 Water holding capacity and oil binding capacity

Hydration or rehydration is the first and perhaps most critical step in imparting desirable functional properties to proteins in a food system. Water retention is defined as the ability of the food material to hold water against gravity [93]. Water holding capacity and oil absorption capacity both were significantly higher in raw peanut protein isolates [16]. Intrinsic factor affecting the water binding capacity of food proteins includes amino acid composition, protein conformation, and surface polarity/hydrophobicity [94]. The water retention capacity is the sum of bound, hydrodynamic and physically entrapped water [95]. During roasting, peanut proteins were denatured by high temperature, exposing more hydrophobic sites, which explained the reduced water retention of peanut protein [96]. With respect to water-holding capacity, the denatured proteins bind more water through exposure of hydrophilic groups [97].

Oil binding capacity and water holding capacity are increased by high pressure treatment [91]. Both properties are important for food texture and flavor. They are high in raw peanut proteins but affected by high temperatures.

6. Role in hunger management

The peanut protein isolates with improved functional food properties are critically needed in many developing countries, because animal protein is more expensive and is getting beyond the reach of many people in developing countries. Abundant proteins in peanuts are cheaper sources of proteins that would serve the purpose. Research data show that peanut and peanut butter consumption improved the feeling of fullness and satisfied the consumers better than the carbohydrates snacks like rice cakes in equal quantities [98]. Another study has shown that peanut consumption can curb the appetites due its fullness effect [99]. Evidence has emerged in favor of type of healthy monounsaturated fat in peanuts that may stimulate a hormone which helps a person to feel satisfied after consumption [100]. Apart from this, it has been seen that daily nutrition peanut consumption leads to long term health benefits. Compared to well-known foods like green tea and red wine, peanuts have higher antioxidant capacity [101].

Groundnut-based 'Plumpy'nut, a ready to use therapeutic food, has helped save the lives of thousands of malnourished children in Niger, by UNICEF [102].

Recent research studies suggest that boiling enhances antioxidant concentration in the peanuts. It has been found that boiled peanuts have 2–4 folds increase in isoflavone antioxidants biochanin A and genistein content, respectively [103].

7. Methods to improve nutrition and manage allergenic properties

Seed storage peanut proteins (such as Ara h 3 and Ara h 4) are less severe in allergenicity compared to their vicilin (Ara h 1) and conglutin (Ara h 2) type seed storage proteins [104–107].

Many methods are tried in peanut protein extracts to reduce their allergenic effect. Among them, roasting, boiling or another heat treatments are most common and require less labour and effort. Though heat treatments sometimes

affect secondary antioxidants due to Maillard reaction products [108]. Some novel processing approaches such as high-pressure processing, pulsed ultraviolet light, high intensity ultrasound, irradiation, and pulsed electric field have been performed toward reducing the immunoreactivity of peanut. Covalent and non-covalent chemical modifications to proteins also have the tendency to alter peanut allergenicity.

The heat or plasma treatment has shown to reduce allergenicity. Roasting lowered allergenicity by 600–700 fold than in native form. The autoclaving decreased immunoreactivity by 50 folds. Among the chemical methods used, it was found that tannic acid (1–2 mg/ml) reduced allergenicity [109]. But it hampers protein digestibility. Use of magnetic beads has also been shown to that it covalently attaches to phenolics.

Conventional Breeding has been reported and varieties missing in isoforms of Ara h 2 or 3 were crossed. Some lines lacking in both Ara h 2 and Ara h 3 were produced [110]. In conventional breeding, large seeded varieties suitable for food have been conventionally bred such as Asha (ICGV 86564) and Namnama (ICGV 90320) in the Philippines [111]. Groundnuts are bred for high oleic to linoleic ratio (O/L ratio) to improve the oil quality. Gorbet and Knauft registered the first high oleic line, SunOleic 95R [112], and it was followed by another variety, Hull with high O/L ratio and resistance to TSWV [113].

Heavy ion beam radiations H1B1 has been used (100 Gy amount) to generate some hypoallergic mutants [114]. Though irradiation affected the production of bioactive compounds. Chung and Champagne [115] have treated protein extract from roasted raw peanuts with POD (peroxidase) and TGA (transglutanase) at 37 °C. They found TGA was not effective but POD was effective for Ara h 1/ Ara h 1 reduction. Different enzyme treatments of alcalase and flavourzyme in Ara h 1, 2 and Ara h 3 have also been done [116]. Pepsin, trypsin and chymotrypsin digestion has also been implemented to reduce allergenicity [117, 118].

RNA interference or genetic engineering techniques have also been suggested to remove allergenic groups of proteins when diversity of seed proteins was analyzed in *Arachis hypogaea* and related species [20]. Three independent transgenic lines have been generated for Ara h 1 and Ara h 6 proteins by using RNA interference [119, 120].

8. Conclusions

The high energy, protein and carbohydrate contents suggest that groundnut or peanut could be of great importance in alleviating protein energy malnutrition and hunger. The minerals analyzed in groundnut were similar to those of other nutritious foods consumed globally, the good levels of fatty acids and amino acids which make them a healthy food for human, and animal nutrition. The low levels of anti-nutrients could enhance absorption of nutrient in groundnut. Also, the functional properties can be modified by simply boiling or roasting and different methods of processing such as heat, pressure, membrane filtering, use of conjugates etc. Though the efforts are ongoing to reduce allergenicity which is generally found more in 2S albumin components and very less in globulins, much research is needed to generate hypoallergic cultivars. On the other hand, peanuts are a rich source of medicinally important phytochemicals of diverse nature. Due to this reason peanut cultivation in developing countries can benefit local communities. Various studies have also increasingly linked peanut consumption with improved human health and with decreased risks of life threatening diseases.

9. Future prospects

Peanut seed storage proteins can be used for different food and feed purposes, and also to make peanut protein biopeptides, hydrolysates, protein films etc. These have variety of industrial applications. In future, research should be aimed at modifying or improving the functional properties and nutritional chemistry to generate food end products. It has been well established with number of studies, that peanut can meet the increasing demand for protein rich healthy food with several benefits and thus, awareness should be spread in many more countries to exploit peanut or groundnut as vegan source of protein. Moreover, new cultivars need to be developed with hypoallergic proteins and improved nutrition.

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Chapter 9

RNAi-Mutants of *Sorghum bicolor* (L.) Moench with Improved Digestibility of Seed Storage Proteins

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Abstract

Modification of the composition of grain storage proteins is an intensively developing area of plant biotechnology, which is of particular importance for sorghum – high-yielding drought tolerant crop. Compared to other cereals, the majority of sorghum cultivars and hybrids are characterized by reduced nutritional value that is caused by a low content of essential amino acids in the seed storage proteins (kafirins), and resistance of kafirins to protease digestion. RNA interference (RNAi) by suppressing synthesis of individual kafirin subclasses may be an effective approach to solve this problem. In this chapter, we review published reports on RNAi silencing of the kafirin-encoding genes. In addition, we present new experimental data on phenotypic effects of RNAi-silencing of *γ-KAFIRIN-1* gene in sorghum cv. Avans. To obtain RNAi mutants with *γ-KAFIRIN-1* gene silencing we used *Agrobacterium*-mediated genetic transformation. Transgenic kernels had modified endosperm type with reduced vitreous layer and significantly improved *in vitro* protein digestibility (93% vs. 57%, according to the densitometry of SDS-PAGE patterns). SDS-PAGE of transgenic kernels showed lowered level of kafirins and appearance of globulin proteins, which were not observed in the original cultivar. For the first time, the cases of instability of inserted genetic construct were identified: elimination of *ubi1*-intron that is a constituent part of the genetic construct for RNAi silencing, or *nos*-promotor governing expression of the marker gene (*bar*) (in the RNAi mutants of cv. Zheltozernoe 10). The research findings presented in this chapter provide strong evidence that RNA interference can be used for improvement of the nutritional properties of sorghum grain.

Keywords: kafirins, *in vitro* protein digestibility, RNAi-mutants, endosperm, *Sorghum bicolor* (L.) Moench

1. Introduction

Grain sorghum is one of the most promising and relatively poorly studied agricultural crops. With its high drought tolerance, sorghum is capable of producing high grain yields in conditions of minimal moisture supply. This crop is of special

importance in the regions regularly exposed to drought, where the stable production of traditional cereals – wheat, maize, barley – is challenging. Moreover, due to the global warming of climate the importance of this crop will steadily increase. Sorghum is already one of the five most important cereal crops cultivated on the Earth. In addition, sorghum grain is gluten-free and can serve as a source of protein for people with celiac disease who are forced to follow a gluten-free diet.

At the same time, compared with other cereals, sorghum grain has a number of significant disadvantages: its storage proteins (kafirins), the content of which reaches 14–16% in some lines and varieties, are poorly digestible by proteases (pepsin, trypsin) [1–4]. The resistance of kafirins to proteolytic digestion reduces the digestibility of starch, which accumulates in significant amounts in sorghum grain (up to 70–75%) since undigested proteins reduce the availability of amylolytic enzymes to starch grains [3, 5, 6]. In addition, the kafirins have low content of indispensible amino acids – lysine, threonine, and tryptophan – and therefore are characterized by low nutritional value [7, 8]. In this regard, increasing the functionality of proteins in sorghum grain, improving their nutritional value is a very urgent problem that has both applied and fundamental importance.

The resistance of kafirins to proteolytic digestion is caused by several factors [9, 10]. Among them are the chemical composition of kafirins, some of which (γ- and β-kafirins) are abundant with sulfur-containing amino acids capable of forming intra- and intermolecular disulfide bonds, hardening protein molecules, and promoting the formation of oligo- and polymers resistant to protease digestion; interaction of kafirins with non-kafirin proteins and non-protein components, in particular, with tannins, which reduce the proteases activity, and with polysaccharides of starch grains; spatial organization of different kafirins in protein bodies of endosperm cells. It was hypothesized that γ-kafirin, which occupies the outer layer of protein bodies and which is the most resistant to proteolytic digestion, prevents the digestion of the α-kafirins – main storage proteins, located inside the protein bodies [11].

An important argument in favor of this hypothesis was the data obtained in the study of the P721Q mutant, induced by chemical mutagenesis and characterized by increased digestibility of kafirins, and the lines derived from this mutant [12, 13]. In this mutant, the protein bodies of endosperm cells have an irregular shape with invaginations. Moreover, γ-kafirin was located only at the bottom of such invaginations, without forming a continuous layer that impedes the access of proteases to α-kafirins [11, 13]. This mutation leads to the formation of kernels with a floury type of endosperm and an increased lysine content, and therefore was denoted with the symbol *hdhl* (*high digestibility high lysine*). Subsequent studies, however, revealed that the P721Q mutant has a point mutation in the signal sequence of one of the 10 copies of the gene encoding the 22 kDa α-kafirin [14]. This sequence is responsible for the packaging of α-kafirin inside the protein body. It was hypothesized that this mutation decreases the accumulation of α-kafirin in protein bodies that leads to a change in their ultrastructure and increases their sensitivity to the action of proteases [14].

To solve the problem of poor digestibility of kafirins various genetic and biotechnological approaches may be used: experimental induction of mutants with impaired synthesis or altered amino acid composition of kafirins [15]; identification of naturally occurring allelic variants of kafirins [16–19]; obtaining transgenic plants with the genetic constructs that induce silencing of γ- and/or α-kafirin genes [20–23]; editing the nucleotide sequences of kafirin genes in order to obtain lines with complete or partial knockout of these genes [24].

RNA interference (RNAi) technology is an effective genetic tool for gene silencing that was used to obtain metabolically engineered plants with improved virus

resistance, starch and oil content, and health benefits in different agriculturally important crops [25–28]. The proposed RNA silencing mechanism starts with the production of 20 to 25 bp small interfering RNAs (siRNAs), which are produced from genetic constructs encoding hairpin RNAs (hpRNA). A typical hpRNA construct is comprised of a sense and an antisense sequence of a portion of target gene mRNA as inverted repeats, and these inverted repeats are separated by a non-complementary spacer region. In most genetic constructs, a spliceable intron is used as spacer because it significantly improves RNA silencing efficiency in plants [29]. The sense and antisense sequences in the transcribed RNA are complementary to each other and form a hpRNA, which is processed by Dicer-like proteins (DCL). The DCL proteins generate siRNAs from a hpRNA precursor. One strand of the siRNA duplex is incorporated into an Argonaute (AGO) protein forming an RNA-induced silencing complex (RISC). The siRNA molecule guides the RISC to the complementary region of single-stranded RNA, and the AGO protein then cleaves the target mRNA.

RNA interference technology has been intensively used to suppress the synthesis of seed storage proteins in different crops including wheat, rice and maize (for review see: [30]). These experiments contributed to obtaining new information on the mechanisms of protein body formation, as well as the role of various classes of prolamins and glutenins in the development of endosperm and the technological properties of flour and dough.

The purpose of our investigations was to obtain the grain sorghum lines with improved digestibility of kafirins using RNA interference technology by

Name	Structure of genetic construction	Reference
pABS032	Maize 19-kDa α-zein promoter; inverted repeats of gene fragments encoding α-A1 (25kDa), α-B1 (19kDa), α-B2 (22kDa), γ1 (27 kDa), γ2 (50 kDa) and δ2 (15 kDa) kafirins, and lysine α-ketoglutarate reductase, separated by the intron of the *ADH1* gene	[20, 34, 35]
pABS166	Maize 19-kDa α-zein promoter; inverted repeats of gene fragments encoding α1 (25 kDa) and γ1 (27 kDa), separated by an intron of the *ADH1* gene	[20, 34, 35]
pABS149	Maize 19-kDa α-zein promoter; inverted repeats of gene fragments encoding γ1 (27 kDa), γ2 (50 kDa), and δ2 (15 kDa) kafirins, lysine α-ketoglutarate reductase, separated by an intron of the *ADH1* gene	[20, 34, 35]
pPTN915	γ-kafirin promoter; complete sequence of the γ-kafirin-1 gene (GeneBank acc. no. X62480), the sequence of the ribozyme gene of the tobacco mosaic virus as a terminator	[21]
pPTN1017	α-kafirin gene promoter; inverted repeats of the α-kafirin (29 kDa) gene fragment, separated by the intron of the Arabidopsis gene encoding the spliceosome D1 protein	[21]
pABS042	Maize 19-kDa α-zein promoter; inverted repeats of δ-kafirin 2 (18 kDa), γ-kafirin 1 (25 kDa), γ-kafirin 2 (50 kDa), and lysine α-ketoglutarate reductase gene fragments, separated by an intron of the alcohol dehydrogenase gene (*ADH1*)	[22]
pABS044	Maize 19-kDa α-zein promoter; inverted repeats of δ-kafirin 2 (18 kDa), γ-kafirin 1 (25 kDa), γ-kafirin 2 (50 kDa), α-kafirin-A1, and lysine α-ketoglutarate reductase gene fragments, separated by an intron of the alcohol dehydrogenase gene (*ADH1*)	[22, 35]
pNRKAF	*35S* promoter; inverted repeats of the γ-kafirin 1 gene fragment (GeneBank accession no. M73688), separated by the maize *ubi1*-intron	[23, 35]

Table 1.
Genetic constructs specially designed to induce RNA silencing of kafirin genes. The molecular masses of kafirins are given in accordance with the author's description.

introducing a genetic construct capable to induce *γ-KAFIRIN-1* silencing. For silencing the *γ-KAFIRIN-1* gene we used the construct pNRKAF [23] that consisted of segment of its nucleotide sequence ([31], GeneBank accession no. M73688) in forward and inverted orientation, which was separated by the sequence of the maize *ubi1*-intron. This construct was driven by the *35S* promoter. Such a construct should suppress the expression of the *γ-KAFIRIN-1* gene using RNA interference. A decrease in the level of γ-kafirin should have "stripped" the protein bodies in transgenic plants and facilitated the digestion of α-kafirins.

In this chapter, we describe phenotypic effects of RNAi-silencing of kafirin genes in two sorghum cultivars – Zheltozernoe-10 (Zh10) and Avans, which contain pNRKAF genetic construct introduced by agrobacterial transformation, as well as characteristic features of other sorghum lines carrying similar genetic constructs for silencing kafirin genes created by other research groups (**Table 1**).

2. Decreased content of kafirins

The primary effect of the functioning of genetic constructs for RNA silencing of kafirin genes is a decreased level of transcripts of these genes. Such an effect was shown for the pPTN915 genetic construct, designed to suppress the expression of

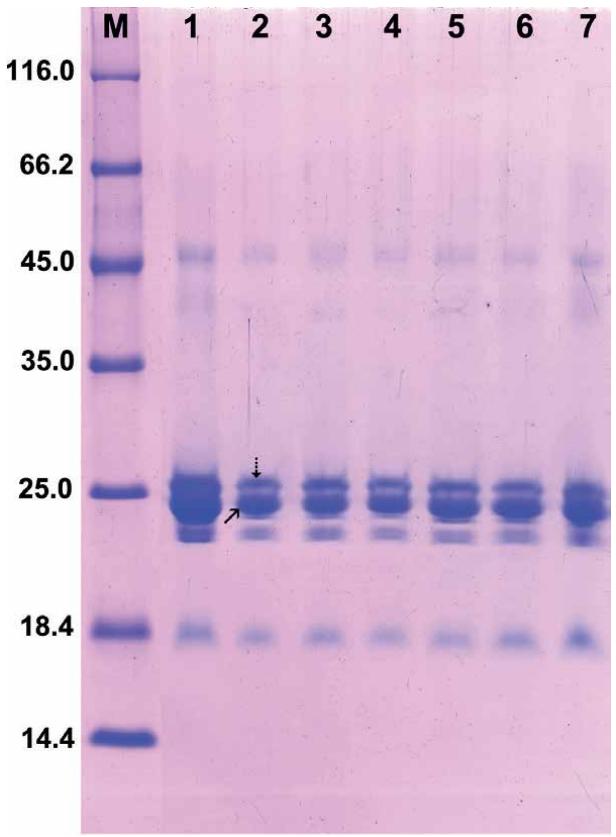

Figure 1.
SDS-PAGE of kafirins from kernels of transgenic plants from the T_1 generation of the RNAi mutant, cv. Avans, isolated under reducing conditions (with the addition of 2-mercaptoethanol). 1 – Original non-transgenic cv. Avans; 2–7 – Individual plants from the T_1 family: 2–6 – Plants with a floury endosperm, containing ubi1-intron; 7 – Plant with a vitreous endosperm, not containing ubi1-intron; M – Molecular mass markers. Kafirins were extracted according to [20]. The arrow marks α-kafirin; the dotted arrow marks γ-kafirin.

the γ-kafirin gene [21]. Many studies using SDS-PAGE have also clearly demonstrated a decrease in the content of monomers and polymers of kafirins [20, 22, 23, 32]. In our experiments, SDS-PAGE of proteins extracted from kernels of transgenic plants of Zh10 in non-reducing conditions (without the addition of 2-mercaptoethanol, which breaks the S-S bonds and, thereby, destroys the polymers of the kafirins), showed a decreased content of γ-kafirin monomer (28 kDa), as well as 47 and 66 kDa oligomers, which are supposed to arise as a result of γ-kafirin polymerization [33]. SDS-PAGE of kafirins extracted under reducing conditions from the kernels of transgenic plants of the Avans cultivar (T_1 generation) carrying the same genetic construct revealed also a noticeable decrease in the content of γ- and α-kafirins (**Figure 1**).

3. Improvement of *in vitro* protein digestibility

The main goal of experiments on silencing of kafirin genes is to improve seed storage protein digestibility. Herewith, depending on the structure of the genetic construct, suppression of certain subclasses of kafirins, and the cultivars used in experiments, the level of digestibility varied significantly.

For example, transgenic plants of cv. Tx430 carrying the ABS166 genetic construct containing inverted repeats of several kafirin genes (α, γ, δ) separated by the intron sequence of the alcohol dehydrogenase gene (*ADH1*) and controlled by the 19-kDa α-zein promoter from maize were characterized by improved *in vitro* protein digestibility. Pepsin treatment of the raw flour and flour that underwent the cooking procedure resulted in 78% and 61% digestibility, respectively, while in the non-transgenic control these indicators varied within 40–50% and 34–40%, respectively [34, 35]. The genetic construct for the silencing of δ- and γ-kafirins (ABS149) also improved the digestibility of raw flour, but did not affect the digestibility of the cooked flour.

Subsequently, new transgenic plants were obtained in the sorghum public line P898012 using other genetic constructs ABS042 and ABS044, created during the ABS (Africa Biofortified Sorghum) project [22]. In these plants, an improvement in the digestibility of flour subjected to the cooking procedure was recorded: from 28% in the control to 39% (for the ABS042 construct for silencing γ- and δ-kafirins), and up to 59% (for the ABS044 construct for silencing α-, γ- and δ-kafirins).

Analysis of ultrastructure of protein bodied showed that in transgenic lines with α-kafirin silencing protein bodies were irregular in shape and had invaginations similar to P721Q mutant [34, 35]. In transgenic lines with γ-kafirin silencing, a diameter of protein bodies was reduced in comparison with original non-transgenic line [36]. In addition, in one of the studied lines, 42–1, protein bodies were highly irregular in shape, with deep invaginations present at the periphery, while in the line 42–2, the protein bodies had small peripheral indentations that gave the boundary region a cracked appearance.

In the experiments of T. Kumar et al. [20] the genetic constructs pPTN915 and pPTN1017 designed for the induction of silencing γ- or α-kafirin, respectively, were also introduced into the genome of the Tx430 line through agrobacterial transformation. *In vitro* digestibility of proteins extracted from the flour of transgenic kernels with silencing of γ-kafirin, subjected to cooking procedure, did not differ from the non-transgenic control, while the silencing of α-kafirin by pPTN1017 improved the *in vitro* protein digestibility of flour subjected to cooking.

Transgenic plants of cv. Zh10 obtained in our experiments carrying the genetic construct pNRKAF for silencing *γ-KAFIRIN-1* gene, also had a significantly

improved *in vitro* digestibility of flour proteins [22]. Comparison of electrophoretic spectra before and after pepsin digestion showed that in the transgenic plants the amount of undigested monomers of α-kafirin and total undigested protein was significantly less (1.7–1.9 times) than in the original non-transgenic line. The digestibility level reached 85.4%, while in the original line this value was about 60%. It is noteworthy that in the kernels of transgenic plant No. 94–3-08 (T_2) with a thick vitreous endosperm, the differences in the digestion of kafirins were more pronounced: the amount of undigested monomers was 17.5 times less, and the amount of total undigested protein was 4.7 times less than in the original line, while the level of digestibility reached 92%.

Plants from the T_3 generation inherited the improved digestibility of kafirins. In these plants, kernels had either a modified type of endosperm with reduced vitreous endosperm, or an endosperm with a well-defined vitreous layer. The level of digestibility of endosperm proteins in these plants was 83–90%, significantly higher than that of the original non-transgenic line (**Figure 2**). Apparently, a decrease in the level of γ-kafirin increases the digestibility of α-kafirins. This increase may be due to chemical reasons (decrease in the amount of polymers) and/or physical reasons (changes in the spatial arrangement of α-kafirins in protein bodies, which increase their availability for cleavage by pepsin). The effect of increased digestibility of kafirins was also observed in plants from the T_4 generation; however, in some cases it disappeared, possibly due to the instability of the introduced genetic construct, or due to its silencing (see below).

After experiments with the model cv. Zh10, we set the task of obtaining RNAi mutants with improved digestibility of kafirins in the new commercial cultivar Avans, which is characterized by a number of agronomically valuable traits. The analysis of the *in vitro* digestibility of proteins from kernels that set on one of the transgenic plants (#1–1) obtained by *Agrobacterium*-mediated genetic transformation with the strain carrying pNRKAF genetic construct showed a significantly higher level of digestibility compared to the original non-transgenic cultivar (**Figure 3**) (93% vs. 57%, according

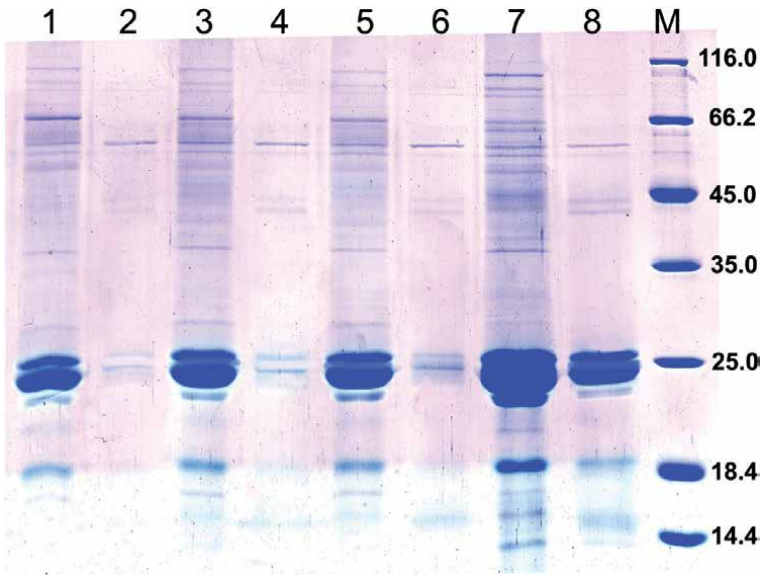

Figure 2.
Electrophoretic spectra of proteins from the flour of transgenic plants from T_3 family #94–3-08 with normal vitreous endosperm. 1–6 – Individual plants from T_3 generation; 7, 8 – Original non-transgenic line Zh10. 1, 3, 5, 7 – Before, 2, 4, 6, 8 – After pepsin digestion. M - molecular mass markers (kDa). [23].

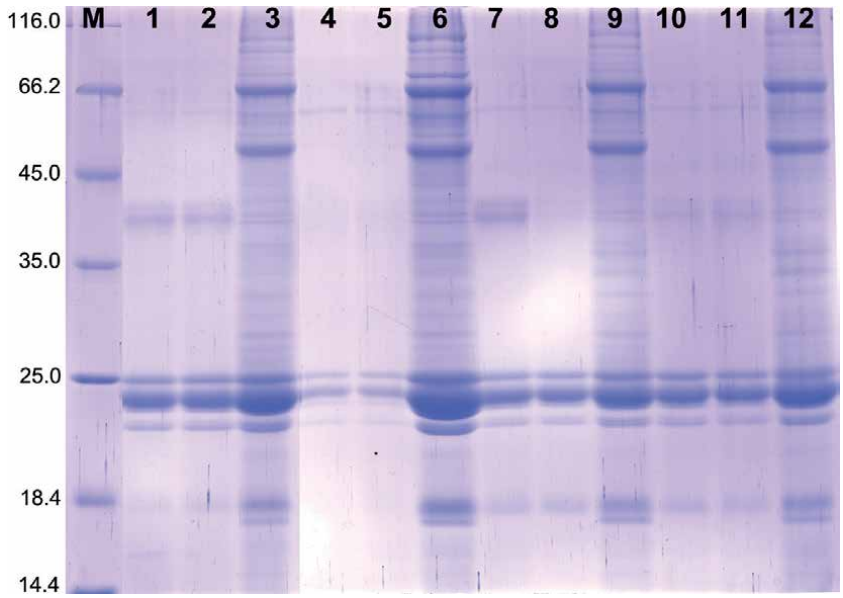

Figure 3.
Electrophoretic spectra of proteins from the flour of sorghum cv. Avans (1–3), transgenic plant #1–1 (4–6) and non-transgenic plants #5–1 (7–9) and #6–4 (10–12). M - molecular mass markers (kDa). 3, 6, 9, 12 – Before, 1, 2, 4, 5, 7, 8, 10, 11 – After pepsin digestion.

to the densitometry of SDS-PAGE patterns). A high level of kafirin digestibility was observed also in the next generation, T_1.

4. Modification of endosperm texture

An important consequence of the functioning of genetic constructs for silencing kafirin genes is a change in the texture of endosperm: in transgenic plants, in most cases, there is complete or partial loss of the vitreous layer, as a result of which the kernels contain only floury endosperm [21, 22, 34, 35]. In our experiments, the

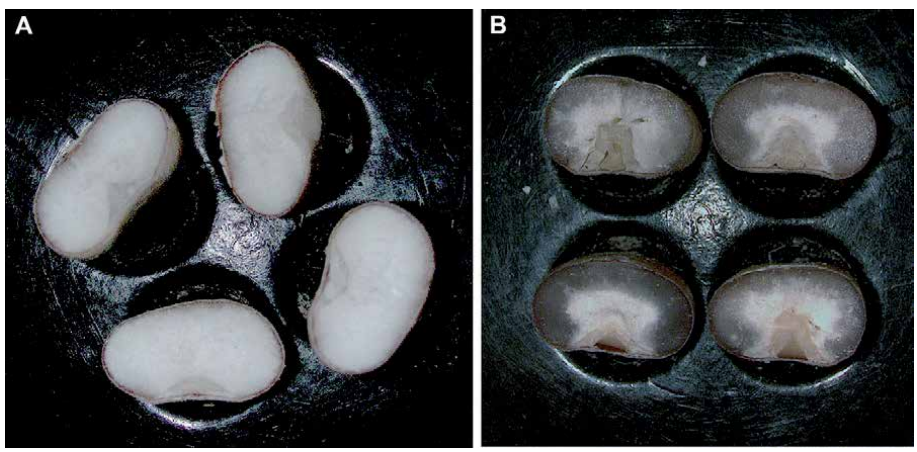

Figure 4.
Cross sections of the kernels of the RNAi mutant #1-1 (A) carrying genetic construct pNRKAF for RNAi silencing, and original cv. Avans (B).

RNAi mutant #1–1 of cv. Avans, had also a floury type of endosperm (**Figure 4**). It should be noted that, in similar experiments in maize, silencing of different zein genes also resulted in reduction of the vitreous endosperm and formation of kernels with floury endosperm [37–39]. It was shown that γ-zein gene plays an important role in the formation of the floury endosperm, and silencing of this gene modified the structure of protein bodies and their connection with starch grains that result in formation of floury endosperm [38].

Unfortunately, the presence of floury endosperm is a significant disadvantage of the obtained lines, since the absence of a vitreous layer increases the fragility of the kernels and reduces its resistance to fungal diseases. It should be noted that the floury (opaque) type of endosperm is characteristic of the P721Q mutant and many

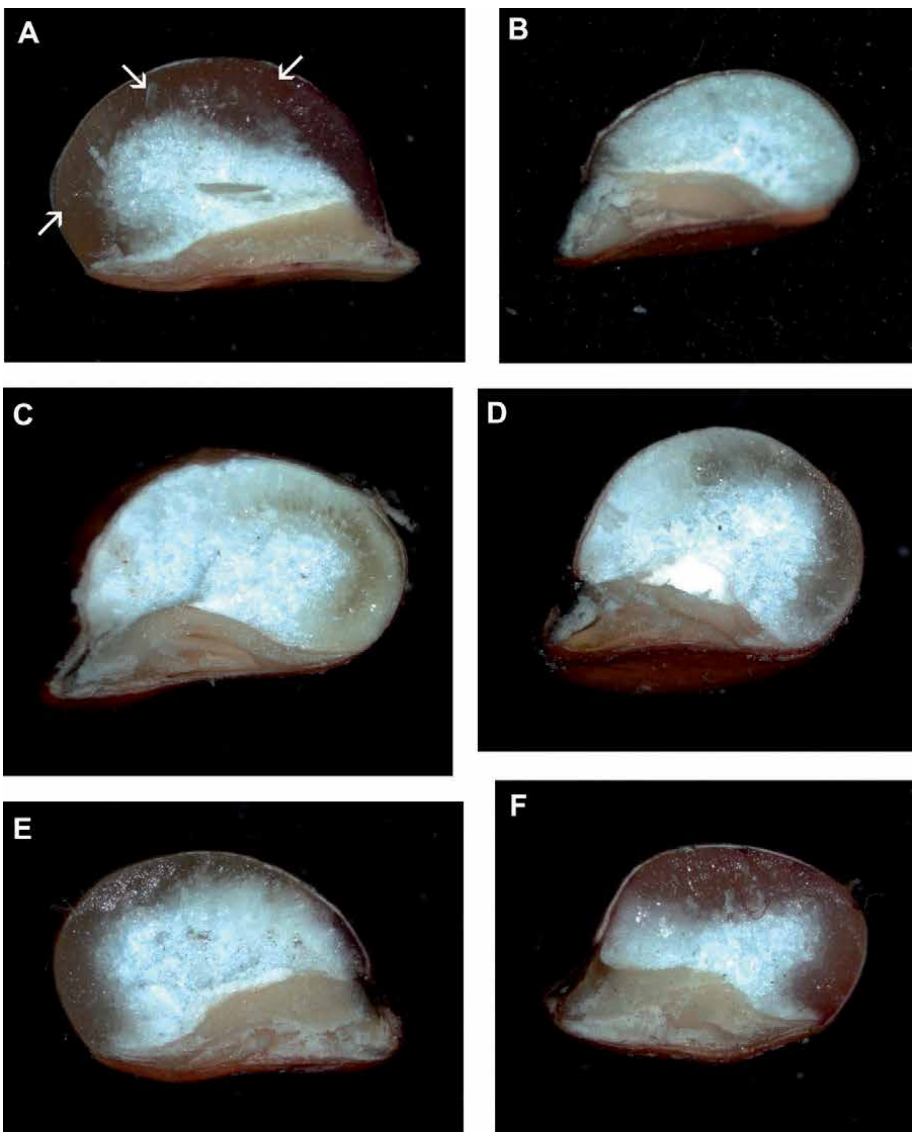

Figure 5.
Longitudinal sections of kernels of the original non-transgenic line Zheltozernoe-10 (A) and transgenic plants carrying pNRKAF genetic construct for RNA silencing of the γ-KAFIRIN-1 gene (B-F), differing in the degree of development of the vitreous endosperm. Vitreous endosperm is marked with white arrows.

breeding lines with improved digestibility of kafirins derived from it. Overcoming this correlation is an extremely difficult and urgent task [8].

In this regard, the transgenic plants of cv. Zh10 with the genetic construction pNRKAF for *γ-KAFIRIN-1* gene silencing are of special interest, since in most cases they had sectors or of the vitreous endosperm in the kernels, or the vitreous endosperm formed a continuous thin layer along the periphery of the kernels (**Figure 5**). It is noteworthy that the formation of the vitreous endosperm in such kernels did not reduce the digestibility of kafirins. Moreover, a plant (94–3-08) was found in T_2, in whose progeny (T_3, kernels is shown in **Figure 5F**) a high level of kafirin digestibility (88–90%) was combined with normal vitreous endosperm [23]. The fact of obtaining such plants shows that an increase in the digestibility of sorghum kafirins may not be associated with the reduction of the vitreous layer and formation of floury endosperm. Further investigation of these plants is needed to understand the role of the γ-kafirin in development of hard endosperm in sorghum.

Previously, transgenic plants with inclusions of vitreous endosperm surrounded by a floury endosperm were also observed in the transgenic plants of cv. Tx430, which contains a genetic construct for silencing α- and γ-kafirins [35]. At the same time, co-suppression of the δ-kafirin and γ-kafirin subclasses did not change the endosperm type in this cultivar. Apparently, the formation of different types of endosperm is due to the peculiarities of the expression of genetic constructs in the genome of the recipient line.

In this regard, it should be noted that the nucleotide sequence that we used in the genetic construct pNRKAF was homologous not only to the γ-*KAFIRIN-1* gene located in the chromosome 2 of the sorghum genome but also to the locus of the chromosome 9 encoding bi-functional protease inhibitor protein (Pfam: PF00234) belonging to the LTP-family (lipid transfer proteins) [23]. It is possible that a higher kafirin digestibility in plant 94–3-08 and its progeny could be due mainly to the suppression of the synthesis of the protease inhibitor, which did not entail a change in the texture of the endosperm.

These data indicate a possible effect of protease inhibitors on the digestibility of proteins in sorghum flour, which remains poorly understood. Purposeful designing of genetic constructs for RNA-silencing of protease inhibitors and their introduction into sorghum genome can help to obtain lines with improved digestibility of kafirins, in which the endosperm could be of the usual vitreous type.

5. Increased synthesis of other proteins

An important consequence of silencing of the prolamine genes in cereals is an increase in the synthesis of other proteins, including those with a higher content of essential amino acids. For example, in transgenic maize plants with α-zein silencing, a double content of tryptophan and lysine was observed [40]. In rice, it was shown that silencing of 13 kDa prolamine increases the total lysine content up to 56% as a result of a compensatory increase in the synthesis of lysine-rich glutelin, globulins, and chaperones [41]. A significant increase in the lysine content (up to 3.3 g / 100 g of protein, compared to 2.1 g/100 g of protein in the non-transgenic control) was found in transgenic sorghum plants carrying complex genetic constructs for RNA silencing of kafirins (ABS032, ABS149) [35]. However, these genetic constructs carried, along with the fragments of the kafirin genes, the fragments of the lysine ketoglutarate reductase gene, which controls the catabolism of free lysine. This fact does not allow drawing a conclusion on the effect of kafirin silencing on the increase in the lysine content in sorghum.

In the transgenic plants obtained in our experiments with a high *in vitro* kafirin digestibility, the total amino acid content in the kernels of plants of the T_2 generation decreased by 22.8–40.2% as compared with the original, non-transgenic line [23]. At the same time, the relative content of the two main essential amino acids, lysine and threonine, has increased significantly. The proportion of lysine increased 1.6–1.7 times: from 1.54% of the total amino acid content in the flour of the original non-transgenic line to 2.41–2.63% in transgenic plants. This increase, combined with a significant decrease in the total level of amino acids, was apparently caused by a decrease in the content of α-kafirins, which are poor in lysine and threonine, while the synthesis of other proteins was not impaired. Accordingly, the relative proportions of lysine and threonine increased. It is possible that suppression of the synthesis of γ-kafirin prevents the accumulation of α-kafirins but does not affect the synthesis of other proteins richer in lysine and threonine. The appearance of new proteins in transgenic sorghum plants carrying a genetic construct for silencing α-kafirin gene was described by T. Kumar et al. [21].

It is noteworthy that in the transgenic plants of the cv. Avans with a construct for silencing γ-*KAFIRIN-1* (RNAi mutant #1–1), along with a decrease in the content of γ- and α-kafirins (**Figure 1**), an increase in the content of a number of globulins occurs, possibly resulting from the re-balancing of the proteome of the kernels (**Figure 6**).

Protein rebalancing in the endosperm is a frequent phenomenon in transgenic plants with genetic constructs for RNA silencing of seed storage proteins. In maize, it was suggested that a compensatory mechanism, which is sensitive to the protein

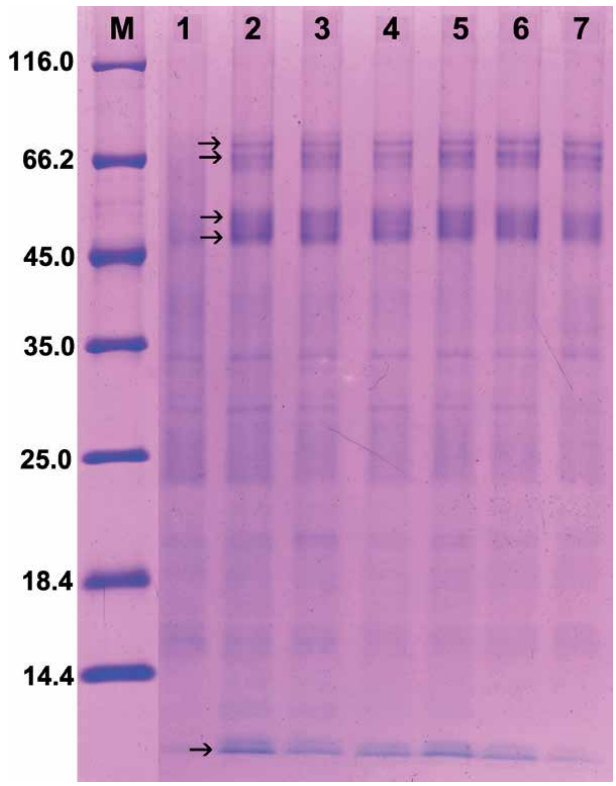

Figure 6.
Electrophoretic spectra of globulins from the kernels of transgenic plants from T_1 generation of RNAi mutant #1–1. 1 – Original non-transgenic cv. Avans; 2–7 individual T_1 plants; M – Molecular mass markers (kDa). Globulins were extracted according to [42].

content exists in the kernels; and a violation of zein synthesis in developing kernels enhances the translation of other mRNAs [43]. It is noteworthy that in transgenic soybean plants with suppressed synthesis of the main storage proteins, the seeds retained an almost identical level of total protein characteristic of untransformed soybean varieties [44]. These data suggest that restoration of proteome balance may be quite common phenomenon, providing a constant supply of nitrogen during seed maturation.

6. Instability of the genetic construct for RNA silencing

In our experiments, we found that the offspring of transgenic plants with a high *in vitro* digestibility of endosperm proteins sometimes lose this trait. Even different panicles of the same plant had different digestibility values. Such instability is an interesting phenomenon, which may be caused by silencing of introduced genetic construct possibly by RNA-dependent DNA methylation that is characteristic to hairpin genetic constructs [45], or by environmental factors, such as temperature, soil moisture, air humidity, etc. It has been reported that temperature causes a significant impact on RNAi-silencing [46]. It was also shown that mRNA degradation induced by microRNA and translation inhibition, depends on the temperature of plant growth [47]. Consequently, the efficiency of inhibition of kafirin synthesis by RNAi-silencing may be sensitive to plant growing conditions, and this was really shown in our experiments [48].

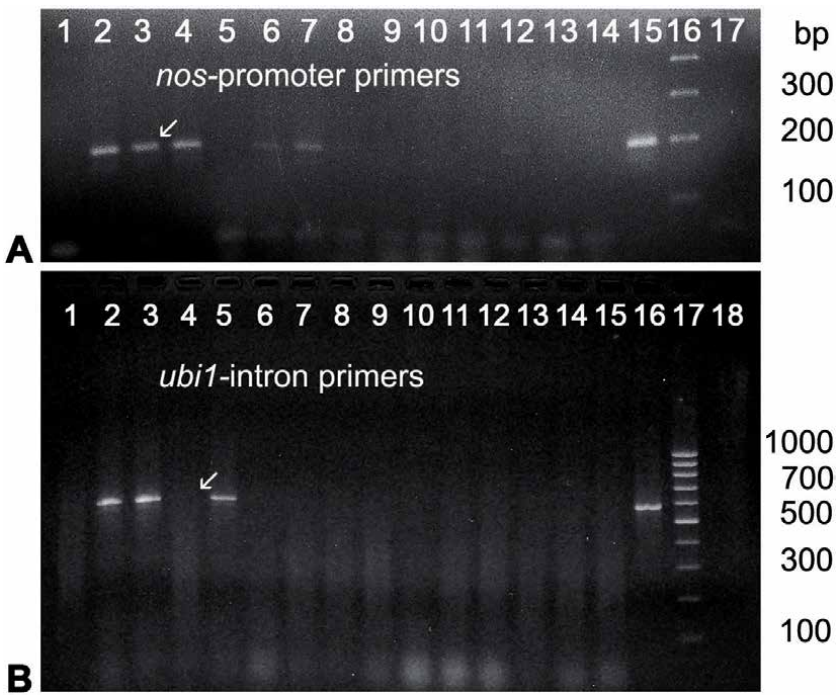

Figure 7.
PCR analysis of plants from the offspring of the RNAi mutant (#1–1, cv. Avans) carrying the genetic construct for silencing γ-KAFIRIN-1, with primers to the nos-promoter (A) and ubi1-intron (B). 1 – Original non-transgenic cv. Avans; 2-4 (A) and 2-5 (B) – individual T_1 plants (A: #2, #3, #4; B: #1, #2, #3, #4, respectively); 5–14 (A) and 6–15 (B) – Plants from another experiment; 15 (A), 16 (B) – A. tumefaciens GV3101/pNRKAF; 16 (A), 17 (B) – DNA markers; 17 (A), 18 (B) – Negative control (no DNA). The nos-specific primers amplified the 202 bp fragment (A). The ubi1-intron specific primers amplified the 588 bp fragment (B). The arrows mark the products of DNA amplification in plant #3.

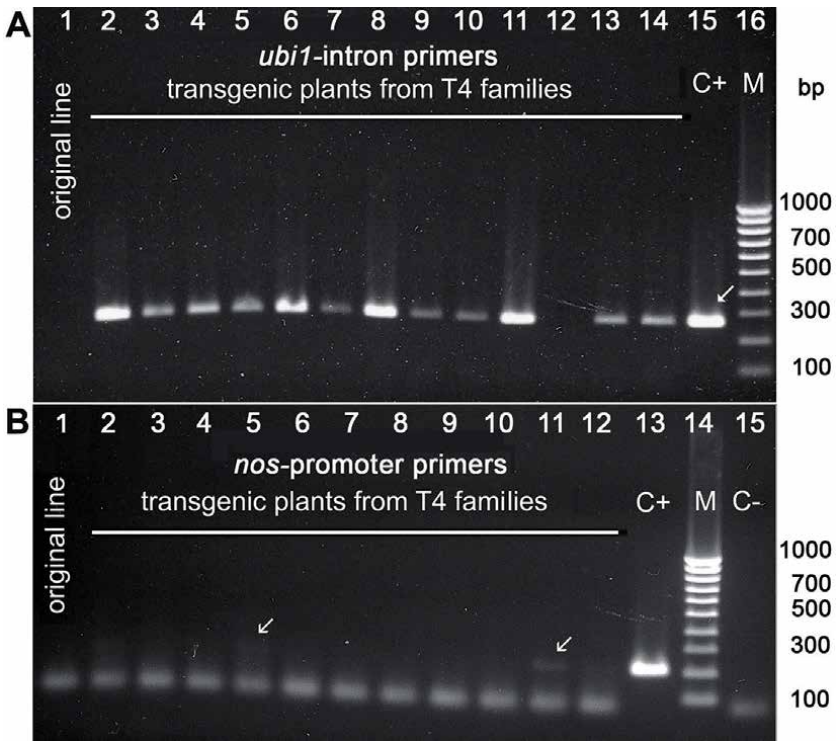

Figure 8.
PCR analysis of transgenic sorghum plants (T_4 generation) carrying a genetic construct pNRKAF [23] with primers to the ubi1-intron (A) and nos-promoter (B). 1 (A, B) – Original non-transgenic line Zh10; 2–-14 (A), 2-12 (B) – DNA of individual transgenic plants from the T_4 families; 15 (A), 14 (B) – A. tumefaciens GV3101/pNRKAF (positive control); 16 (A), 14 (B) – DNA markers; 15 (A) – Negative control (no DNA). The ubi1-intron specific primers amplified the 267 bp fragment (A). The nos-specific primers amplified the 202 bp fragment (B). Amplified gene-specific fragments are marked with arrows [50].

In addition to instability at the epigenetic level, we have found the genetic instability of introduced construct for RNAi-silencing. In this regard, analysis of the progeny of the RNAi mutant #1–1 (cv. Avans), carrying a construct for silencing γ-*KAFIRIN-1*, is indicative. Of the 4 studied T_1 plants grown in the experimental field plot, all plants were transgenic, because carried the *nos*-promoter driving the expression of the marker gene *bar*, located in T-DNA of pNRKAF, along with a genetic construct for the γ-*KAFIRIN-1* gene silencing (**Figure 7A**). At the same time, one of these plants (#3) lacked the *ubi1*-intron, which is a part of the genetic construct for silencing (**Figure 7B**). All kernels developed in the panicle of plant #3 had the vitreous type of endosperm, characteristic to the original cultivar (**Figure 4A**), while in the panicles of other plants, in which the *ubi1*-intron was present, the kernels had a floury type of endosperm (**Figure 4B**), characteristic for transgenic plants with γ-kafirin silencing.

In addition, in Zh10 transgenic plants from the T_4 families with high digestibility of kafirins, probable elimination of the *nos*-promoter, which controls the expression of the marker gene *bar* in the pNRKAF genetic construct [23] was found [49]. **Figure 8** clearly shows that in the plants from the T_4 families, amplification of the *ubi1*-intron fragment was observed, while amplification of the *nos*-promoter located in the construct in front of the marker gene *bar* was absent. Thus, these plants probably turned out to be functionally marker-free transgenic plants. This fact is of significant interest, since the presence of marker genes in the genetic constructs hinders the practical use of transgenic lines in practical plant breeding.

7. Conclusions

The research findings presented in this chapter provide strong evidence that RNA interference can be used for the improvement of the nutritional value of grain sorghum. RNAi mutants are characterized by significantly improved digestibility of kafirins and higher content of essential amino acids, in particular lysine. In some cases, these mutants retain vitreous endosperm that is highly important for grain hardiness and in ensuring the resistance of kernels to fungal diseases.

Nevertheless, in most cases the kernels with suppressed synthesis of γ- or α-kafirins have floury endosperm that strongly reduces their use in sorghum breeding. Such a correlation between the traits of high digestibility of kafirins and the floury type of endosperm, which was originally observed in the P721Q mutant and lines created on its basis is a serious problem (see review [8]). In maize, the correlation between the floury endosperm and the increased lysine content was disrupted using modifier genes that enhanced the accumulation of γ-zein [42, 51, 52]. However, in sorghum, an increase in the synthesis of γ-kafirin may decrease the level of kafirin digestibility due to a high content of sulfur-containing amino acids, which contribute to the polymerization of kafirins. Possibly, one of the ways to solve this problem may be down-regulation of genes that encode protease inhibitors, which can also affect the level of digestion of kafirins by exogenous proteases. In this case, the resulting lines would have a hard endosperm in combination with a high digestibility of kafirins.

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Chapter 10

Characterisation of Endo-Polygalacturonases Activities of Rice (*Oryza sativa*) Fungal Pathogens in Nigeria, West Africa

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Abstract

Rice (*Oryza sativa*) is cultivated in swampy geographical locations of tropical Nigeria, West Africa. Here it is infected by a host of fungal pathogens on the field or contaminated at postharvest. This has led to its loss and reduction in its production in both the national and global market. *Lasiodiplodia theobromae* and *Rhizoctonia solani* have recently been identified as the major fungal phytopathogens causing the deterioration of this grain on the field and at postharvest and affecting its production in Nigeria leading to gross capital loss. Hence the need to determine physiological control measures for the eradication of both phytopathogens on the field and at postharvest. In this study, tropical strains of *Lasiodiplodia theobromae* and *Rhizoctonia solani* obtained from deteriorated rice (*Oryza sativa*) were grown in a growth nutrient medium composed of $MgSo_4.7H_20$, K_2HPO_4, $FeSO_4.7H_20$, potassium nitrate and pectin at 30°C. Endo-Polygalacturonase activities were produced by the fungal isolates in the growth medium within ten days. The endo-polygalacturonases from both fungi were purified by a combination of ammonium sulphate precipitation, dialysis, gel filtration (on Sephadex G-100 column) and ion-exchange chromatography (on CM-Sephadex C-50 and CM-Sephadex C-25 columns). The molecular weight of endo-polygalacturonase from the *Lasiodiplodia theobromae* using Sephadex G-100 was estimated as 124,000 Daltons while that of the *Rhizoctonia solani* was estimated as 92,000 Daltons. The purified endo-polygalcuronase from the *Lasiodiplodia theobromae* exhibited optimum activity at 30°C and at pH 4.5 while that from the *Rhizoctonia solani* exhibited optimum activity at 32°C and at pH 5.0. The purified endo-polygalacturonases from both fungi exhibited optimum activities at 0.2% pectin concentration. They were stimulated by Ca^{2+} but inhibited by ethlylenediamine tetracetic acid (EDTA) and 2,4-dinitrophenol. The purified endo-polygalacturonase from the *Lasiodiplodia theobromae* lost 80% of its activity within 20 minutes of heat at 80°C. While the purified endo-polygalacturonase from the *Rhizoctonia solani* lost 82% of its activity within 20 minutes of heat at 80°C. Potassium nitrate as nitrogen source in the defined growth medium with pectin as carbon source supported highest activity of endo-polygalacturonase by the *Lasiodiplodia theobromae* while ammonium chloride as nitrogen source in the defined growth medium with

pectin as carbon source supported highest activity of endo-polygalacturonase by the *Rhizoctonia solani*. In conclusion, the conditions inhibiting endo-polygalacturonases from *Lasiodiplodia theobromae* and *Rhizoctonia solani* capable of degrading the pectin portion of rice (*Oryza sativa*) can be adapted as feasible control measures limiting the infection and contamination of rice (*Oryza sativa*) by these phytopathogens on the field and at postharvest. Temperature and pH extreme from 30°C and pH 4.5 will be feasible inhibitory control measures for the growth of *Lasiodiplodia theobromae* on rice (*Oryza sativa*) in Nigeria while temperature and pH extreme from 32°C and pH 5.0 will inhibit growth of *Rhizoctonia solani* on the grain. These physiological conditions will preserve pectin in rice (*Oryza sativa*) from degradation by these two fungal phytopathogens.

Keywords: rice (*Oryza sativa*), phytopathogen, fungi, microbial enzymes, polygalacturonase, endo-polygalacturonase, purification, characterisation

1. Introduction

Oryza sativa (Asian rice) and *Oryza glaberrima* (African rice) are species of rice grown all over the world [1]. Rice is the species of the seed of grass. It is a cereal grain consumed mostly in Asia and Africa [2]. It is an agricultural grain which has the third-highest worldwide production only after sugarcane and maize [3, 4] and the world's most consumed staple food [4]. It is rich in starch, protein, minerals and vitamins but low in calories and fats [5]. Rice is grown in all the geographical zones of Nigeria, West Africa depending on the variety [6]. The area of land used for rice cultivation in Nigeria, West Africa is about 2 million hectares. Nigeria however has the potentials of cultivating about 5 million hectares [7, 8].

Rice is affected by a host of fungal pathogens which include: *Magnaporthe grisea* which causes Rice blast; *Rhizoctonia solani* which causes Rice sheath blight; *Cochliobolus miyabeanus* (an Ascomycete) which causes brown spot disease in rice [1]. In Nigeria, the major fungal pathogens of rice include: *Fusarium moniliforme* Sheldon which causes Bakanae foot rot disease; *Cercospora oryzae* Miyake which causes Narrow brown leaf spot; *Rhynchosporium oryzae* Hashioka and *Rhynchosporium oryzae* Yokogi which cause Leaf scald; *Rhizoctonia solani* Kühn which causes Basal sheath rot; *Pyricularia oryzae* Cav. which causes Rice blast; *Cochliobolus miyabeanus* Ito Dreschler ex Dastur and *Cochliobolus miyabeanus* Kuribayashi Dreschler ex Dastur which cause Rice brown spot; *Lasiodiplodia theobromae* which causes Root rot disease complex in rice [9, 10].

Endo-Polygalacturonase (EC: 3.2.1.15) also known as Pectin depolymerase, PG, Pectolase, Pectin hydrolase, and Poly-alpha-1,4-galacturonide glycanohydrolase, is an enzyme that hydrolyzes the alpha-1,4 glycosidic bonds between galacturonic acid residues. It degrades pectin by hydrolyzing the O-glycosyl bonds yielding alpha-1,4-polygalacturonic residues [11–14]. This enzyme has multiple parallel beta sheets which form a helical shape that is called a beta helix. This highly stable structure has numerous hydrogen bonds and disulfide bonds between strands common to all pectin degrading enzymes. The interior of the beta helix is hydrophobic [14, 15]. Exo-Polygalacturonases and Endo-Polygalacturonases have differing hydrolytic modes of action. Endo-Polygalacturonases hydrolyze pectin in a random fashion along the polygalacturonan chain resulting in oligogalacturonides. Exo-Polygalacturonases hydrolyze pectin at the non-reducing end of the polymer resulting in monosaccharide galacturonic acid [14]. Fungal Polygalacturonases

are affected by a variety of factors which include: pH, substrate concentration, substrate specificity, and temperature [13]. Phytopathogenic fungi expose plant cell walls to Cell Wall Degrading Enzymes (CWDEs) such as Polygalacturonases [14]. Siddiqui [16] purified a monomeric polygalacturase with molecular weight of 32 kDa and optimum activity at 55°C and at pH 5.0 using Sephadex G-200 and Sephacryl S-100 from thermophilic *Rhizomucor pusillus* isolated from decomposting orange peels. Anand *et al*. [17] purified an endo-polygalacturonase using acetone precipitation and gel filtration from a strain of *Aspergillus* fumigatus. They determined the molecular weight to be 43.0 kDa, stable at a pH range of 7–10 and with optimum activity at 30°C. The polygalacturonase was stimulated by Cu^{2+} and K^{+} but inhibited by Ag^{+}, Hg^{2+} and Ca^{2+}. Doughari and Onyebarachi [18] produced polygalacturonase from *Aspergillus flavus* isolated from orange peel with maximum activity in the presence of polygalacturonic acid at 35°C and pH 4.5. Carrasco *et al*. [19] reported from their studies the expression of a polygalaturonase in *Pichia pastoris* with an optimum activity 15°C higher than its mesophilic counterpart. According to Thakur [20], *Mucor circinelloides* was able to produce an extracellular polygalacturonase in a growth medium with pectin methyl ester (1% w/v) as carbon source and a combination of casein hydrolysate (0.1% w/v) and yeast extract (0.1% w/v) as nitrogen source. Optimum polygalcturonase activity was obtained at pH 4.0.

The aim of this study was to determine the physiological conditions that will inhibit the growth of major and specific fungal phytopathogens of rice (*Oryza sativa*) in Nigeria, West Africa. The present study will establish the contributions of endo-polygalacyuronases produced by *Lasiodiplodia theobromae* and *Rhizoctonia solani* to the deterioration of rice (*Oryza sativa*) cultivated in Nigeria. Control measures which include conditions inhibiting endo-polygalacturonases from these specific fungal phytopathogens can be adapted in the cultivation (pre-harvest) and storage (post-harvest) of rice (*Oryza sativa*) globally.

2. Materials and methods

2.1 Source and identification of isolates

The tropical fungal strains of *Lasiodiplodia theobromae* and *Rhizoctonia solani* for this investigation were isolated from deteriorated rice (*Oryza sativa*) obtained from Cocoa Research Institute, Ibadan, Nigeria. The isolates were identified at the International Institute of Tropical Agriculture, Ibadan, Nigeria. The isolates were cultured on potato dextrose agar on plates and slants.

2.2 Inocula and their culture conditions

Lasiodiplodia theobromae and *Rhizoctonia solani* isolated from deteriorated rice (*Oryza sativa*) were grown in a fungal growth medium with defined specific nitrogen and carbon sources for growth. The isolates were cultured on plates and slants containing potato dextrose agar at 30°C. Ninety six-hour-old cultures of isolates were used in the investigation [21]. Isolates were cultured in a growth medium composed of $MgSO_4.7H_20$ (0.1 g), K_2HPO_4 (2 g), KH_2PO_4 (0.5 g), $FeSO_4.7H_2O$ (1 mg), KNO_3 (9.9 g) and pectin (10 g) source (Sigma-Aldrich, USA) per 1 litre of distilled water (The pH of the medium was adjusted to pH 5.0 using 0.1 N HCl and 0.1 N NaOH). Conical flasks (250 ml) containing 100 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately 6 x 10^5 spores

per ml of each isolate. These were the experimental flasks. Control flasks contained un-inoculated medium. Experimental and control flasks were incubated without shaking at 30°C. Protein content was determined [22].

2.3 Enzyme extraction

Contents of flasks were carefully filtered through glass fibre filter paper (Whatman GF/A) on the tenth day of inoculation of growth medium. Protein content of the filtrates was determined [22]. The filtrates were also assayed for polygalacturonase activity [23, 24].

2.4 Precipitation using ammonium sulphate

The crude enzymes of the isolates was treated with ammonium sulphate (analytical grade, Sigma) within 40–90% saturation. Precipitation was at 4°C for 24 hours. Centrifugation was done at 4000 rpm for 30 minutes at 4°C using a high speed cold centrifuge. The precipitate was re-constituted in 0.2 M citrate phosphate buffer (pH 5.0). Protein content of the precipitated enzyme was determined [22]. Polygalacturonase activity was also determined [23, 24].

2.5 Dialysis

The ammonium sulphate precipitated enzyme of each isolate was dialyzed using acetylated dialysis tubings (Visking dialysis tubings, Sigma) [25] and a multiple dialyser (Pope Scientific Inc. Model 220, USA). The enzyme preparation was dialyzed using 0.2 M citrate phosphate buffer (pH 5.0) at 4°C for 24 hours under several changes of the buffer. The protein content of the dialyzed enzyme was afterwards determined using the Lowry *et al.* [22] method. Polygalacturonase activity of the dialyzed enzyme was also determined [23, 24].

2.6 Enzyme assay

2.6.1 Endo-polygalacturonase (PG)

Reaction mixture consisted of 1 ml of 0.1% (w/v) pectin in 0.2 M citrate phosphate buffer (pH 5.0) as substrate and 0.5 ml enzyme. Controls consisted of only 1 ml of substrate with no enzyme added. The contents of both experimental and control tubes were incubated at 35°C for 1 hr. The reaction in each tube was terminated with 3 ml of dinitrosalicylic acid reagent (Appendix 1). Thereafter, 0.5 ml of enzyme was added to the controls. The contents of tubes were then boiled for 15 minutes. Optical density readings were taken at 540 nm using a colorimeter. The total reducing sugars released in the reaction mixtures was determined by the Dinitrosalicylate (DNSA) method [23, 24]. One unit of endo-polygalacturonase activity was defined as the amount enzyme in 1 ml of reaction mixture which released reducing sugars equivalent to 100 μg galacturonic acid per minute under specified assay conditions. Specific activity was expressed as enzyme units per mg protein.

2.7 Determination of protein concentration

Using the Lowry *et al*. [22] method, protein concentration was determined at every stage of the investigation. Reaction was with copper in the presence of alkali (Appendix 1).

2.8 Fractionation of enzyme using gel filtration and ion-exchange chromatography

Endo-Polygalacturonase from *Lasiodiplodia theobromae* and *Rhizoctonia solani* were subjected to further purification by gel filtration using a Sephadex G-100 column and ion-exchange chromatogeaphy using CM-Sephadex C-25 and CM-Sephadex C-50 columns. The Sephadex G-100 column was calibrated with proteins of known molecular weight [26, 27].

2.9 Characterisation of the purified endo-polygalacturonases

The effects of temperature, pH, substrate concentrations, certain cations and specific inhibitors on the activity of the purified endo-polygalacturonases from *Lasiodiplodia theobromae* and *Rhizoctonia solani* were investigated.

2.10 Effect of temperature

The substrate used was 0.1% (w/v) pectin (Sigma-Aldrich, USA) in 0.2 M citrate phosphate buffer (pH 5.0). The reaction mixture consisted 1 ml of substrate and 0.5 ml of enzyme. Incubation was at a range of 5-70°C for 1 hr.

2.11 Heat stability test at 80°C

The effect of heat (80°C) on the stability of the purified endo-polygalacturonase at different periods, 5, 10, 15, 20 and 25 minutes was carried out. The activity of the heated endo-polygalacturonase was determined by incubating 0.5 ml of enzyme plus 1 ml of 0.1% (w/v) pectin (Sigma-Aldrich, USA) in citrate phosphate buffer (pH 5.0) substrate at 35°C for 1 hr.

2.12 Effect of pH

The substrate used was 0.1% (w/v) pectin (Sigma-Aldrich, USA) in 0.2 M citrate phosphate buffer at different pH values ranging from pH 4.0–8.5. The reaction mixture consisted 1 ml of substrate and 0.5 ml of enzyme. Incubation was performed at 35°C for 1 hr.

2.13 Effect of concentrations of substrate (pectin)

Concentrations which were 0.05–0.3% (w/v) pectin (Sigma-Aldrich, USA) constituted in 0.2 M citrate phosphate buffer (pH 5.0) were used as substrate in this investigation. The reaction mixture consisted 1 ml of substrate and 0.5 ml of enzyme, incubated at 35°C for 1 hr.

2.14 Effects of cations

The cations NaCl and $CaCl_2$ were used at different concentrations (5, 10, 15, 20 and 30 mM) for the activity of the purified endo-polygalacturonase. Each cation was constituted in 0.1% pectin in citrate phosphate buffer (pH 5.0. The reaction mixture was 1 ml of substrate and 0.5 ml of enzyme incubated at 35°C for 1 hr.

2.15 Effects of certain inhibitors

2,4-Dinitrophenol and ethylenediamine tetraacetic acid were the inhibitors used in this investigation. They were prepared at concentrations of 2, 4, 6, 8 and 10 mM

in 0.1% pectin (Sigma-Aldrich, USA) in citrate phosphate buffer at pH 5.0. These were the substrates.

2.16 Effects of specific nitrogenous compounds

The nitrogenous compounds used were potassium nitrate, ammonium sulphate and ammonium chloride. Pectin was the constant carbon source in the growth medium. Endo-polygalacturonase activity expressed by the fungal strains of *Lasiodiplodia theobromae* and *Rhizoctonia solani* were investigated. Endo-Polygalacturonase activity expressed on the tenth day of incubation at 30°C was recorded.

3. Results

When strains of *Lasiodiplodia theobromae* and *Rhizoctonia solani* isolated from deteriorated rice (*Oryza sativa*) were grown in a defined growth medium containing potassium nitrate as nitrogen source and pectin as carbon source, they expressed endo-polygalacturonase activities at 30°C within ten days. The enzymes were isolated and purified by ammonium sulphate precipitation, gel filtration and ion-exchange chromatography. The purification steps are presented on **Tables 1** and **2**. Purification of the endo-polygalacturonase from the *Lasiodiplodia theobromae* by gel filtration using Sephadex G-100 gave four peaks of absorption designated A, B, C. Only the fractions with peak B expressed endo-polygalacturonase activity. The molecular weight estimate of endo-polyglacturonase produced by the *Lasiodiplodia theobromae* was 124,000 Daltons. Purification of the endo-polygalacturonase from *Rhizoctonia solani* by gel filtration (Sephadex G-100) gave two peaks of absorption designated D and E. Only the fractions of peak E expressed endo-polygalacturonase activity. The molecular weight estimate of endo-polygalacturonase from *Rhizoctonia solani* was 92,000 Daltons.

Fraction	Total Activity (Units)	Total Protein (mg)	Specific Activity (Units/mg protein)	Yield (%)	Purification fold
Crude extract	4120	60.2	68.4	100	1
90% $(NH_4)_2SO_4$ Precipitation	3228	40.3	80.0	78.3	1.16
Sephadex G-100 Gel filtration Chromatography					
Peak A	2139	10.6	201.79	51.9	2.95
Peak B	1023	8.3	123.25	24.8	1.80
Peak C	995	4.6	216.3	24.1	3.16
CM-Sephadex C-50 Ion-Exchange Chromatography					
Peak Ba	966	2.8	345	23.4	5.04

Table 1.
Purification of endo-polygalacturonase from Lasiodiplodia theobromae *isolated from deteriorated rice (*Oryza sativa*).*

Fraction	Total Activity (Units)	Total Protein (mg)	Specific Activity (Units/mg protein)	Yield (%)	Purification fold
Crude extract	1615	32.2	50.1	100	1
90% $(NH_4)_2SO_4$ Precipitation	1328	22.1	60.1	82.2	1.2
Sephadex G-100 Gel filtration Chromatography					
Peak D	1121	3.1	361.6	69.4	7.2
Peak E	926	7.4	125.1	57.3	2.5
CM-Sephadex C-25 Ion-Exchange Chromatography					
Peak Eb	895	6.5	137.7	55.4	2.7

Table 2.
Purification of endo-polygalacturonase from Rhizoctonia solani *isolated from deteriorated rice (*Oryza sativa*).*

The purified endo-polygalcuronase from the *Lasiodiplodia theobromae* (ion-exchange chromatographic fraction - CM-Sephadex C-50) exhibited optimum activity at 30°C and at pH 4.5 while the purified endo-polygalacturonase from the *Rhizoctonia solani* (ion-exchange chromatographic fractions - CM-Sephadex C-25) exhibited optimum activity at 32°C and at pH 5.0.

The purified endo-polygalacturonases from both the *Lasiodiplodia theobromae* and the *Rhizoctonia solani* exhibited optimum activities at 0.2% pectin concentration.

The purified endo-polygalacturonases from both fungi were stimulated by Ca^{2+} They were inhibited by ethlylenediamine tetracetic acid (EDTA) and 2,4-dinitrophenol.

The purified endo-polygalacturonase from the *Lasiodiplodia theobromae* lost 80% of its activity within 20 minutes of heat at 80°C. While the purified endo-polygalacturonase from the *Rhizoctonia solani* lost 82% of its activity within 20 minutes of heat at 80°C.

Potassium nitrate as nitrogen source in the defined growth medium with pectin as carbon source supported highest activity of endo-polygalacturonase by the *Lasiodiplodia theobromae* however, ammonium chloride as nitrogen source in the defined growth medium with pectin as carbon source supported highest activity of endo-polygalacturonase by the *Rhizoctonia solani*.

4. Discussion

Pectin is found in the tissues of rice (*Oryza sativa*) [28]. In this investigation, we observed that the fungal pathogens *Lasiodiplodia theobromae* and *Rhizoctonia solani* isolated from deteriorated rice (*Oryza sativa*) produced endo-polygalacturonases in a growth medium containing pectin as carbon source and potassium nitrate as nitrogen source of fungal growth at 30°C. In Nigeria, West Africa, *Lasiodiplodia theobromae* and *Rhizoctonia solani* are fungal pathogens of rice (*Oryza sativa*) in the field [9, 10, 29]. From the results of this study, we can therefore establish that *Lasiodiplodia theobromae* and *Rhizoctonia solani* are capable of causing the deterioration of rice (*Oryza sativa*) at 30°C by breaking down the grain's pectin portion.

In this research, we observed that the molecular weight of endo-polygalacturonase from our strain of *Lasiodiplodia theobromae* using Sephadex G-100 has an estimate of 124,000 Daltons while that of our strain of *Rhizoctonia solani* has an estimate of 92,000 Daltons. Adejuwon *et al.* [11] reported from their studies a polygalacturonase from *Penicillium funiculosum* Thom. isolated from tomato fruits with molecular weight estimate of 89,100 Daltons. Ajayi *et al*. [12] in their own investigation purified polygalacturonase from *Rhizopus arrhizus* Fisher isolated from tomato fruits with molecular weight estimates of approximately 166,000 Daltons and 60,260 Daltons. Olutiola [13] reported from his investigation, an extracellular polyglacturonase complex from *Penicillium citrinum* associated with internal mouldiness of cocoa (*Theobroma cacao*) beans having molecular weights of 30,000 and 56,000.

The purified endo-polygalcuronase from our *Lasiodiplodia theobromae* exhibited optimum activity at 30°C and at pH 4.5 while that from our *Rhizoctonia solani* exhibited optimum activity at 32°C and at pH 5.0. Olutiola [30] reported that *Penicillium sclerotigenum* Yamamoto isolated from rotten yam tuber produced a polygalacturonase with optimum activity at pH 5.0.

The purified endo-polygalacturonases from both fungi exhibited optimum activities at 0.2% pectin concentration. The purified endo-polygalacturonases from both fungi were stimulated by Ca^{2+} but inhibited by ethlylenediamine tetracetic acid (EDTA) and 2,4-dinitrophenol. Ajayi *et al.* [31] purified polygalacturonase from *Rhizopus arrhizus* Fisher with optimum activity at pH 4.5, stimulated by Ca^{2+} but inhibited by ethlylenediamine tetracetic acid (EDTA) and 2,4-dinitrophenol. The purified endo-polygalacturonase from our *Lasiodiplodia theobromae* lost 80% of its activity within 20 minutes of heat at 80°C. While the purified endo-polygalacturonase from our *Rhizoctonia solani* lost 82% of its activity within 20 minutes of heat at 80°C. Potassium nitrate as nitrogen source in the defined growth medium with pectin as carbon source supported highest activity of endo-polygalacturonase by *Lasiodiplodia theobromae* while ammonium chloride as nitrogen source in the defined growth medium with pectin as carbon source supported highest activity of endo-polygalacturonase by *Rhizoctonia solani*. According to Olutiola [32], pectin as carbon source with L-asparagine as nitrogen source of a defined growth medium supported the growth and sporulation of *Fusarium oxysporum* Schlecht. In conclusion, the conditions inhibiting endo-polygalacturonases from *Lasiodiplodia theobromae* and *Rhizoctonia solani* capable of degrading the pectin portion of rice (*Oryza sativa*) observed in this study can be adapted as feasible control measures limiting the infection and contamination of rice (*Oryza sativa*) by these phytopathogens on the field and at postharvest. Temperature and pH extreme from 30°C and pH 4.5 will be feasible inhibitory control measures for the growth of *Lasiodiplodia theobromae* on rice (*Oryza sativa*) in Nigeria and particularly the preservation of pectin in rice (*Oryza sativa*) from degradation by this particular phytopathogen. Temperature and pH extreme from 32°C and pH 5.0 will inhibit growth of *Rhizoctonia solani* on rice (*Oryza sativa*) and preserve pectin in this grain from degradation by *Rhizoctonia solani*.

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Conflict of interest

Authors declare no conflict of interest.

A. Appendix

A.1 DNSA reagents for terminating endo-polygalacturonase activity

Reagents [17]

i. Sodium hydroxide – 10 g

ii. Potassium sodium tartrate – 200 g

iii. Phenol – 2 g

iv. 3, 5-Dinitrosalicylic acid – 10 g

v. 5% Sodium sulphite – 10 ml

vi. 0.03% Glucose – 10 ml

vii. Distilled water – 1Litre

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Chapter 11

Impact of Inadequate Concentration of Boron in Seed Storage Proteins Content in Oilseed Crops

Archana, Preetam Verma and Nalini Pandey

Abstract

For the estimation of Impact of inadequate concentration of boron in seed storage proteins content in oilseed crops, a sand culture experiment was designed and all the three crops i.e. soyabean, mustard and linseed were grown under sufficient and insufficient boron treatment till maturity. Seed germination and seed storage protein concentration was determined in seeds after the harvesting of crops. Earlier oilseed crops like soyabean, mustard and linseed are cultivated for oil production but at this time these crops are reliable source of protein also and are real asset for human dietary protein. The storage protein present in seeds varies from ~10% (in cereals) to 40% (in certain legumes and oilseeds) of dry weight. Seeds contain one or more groups of proteins that are present in high amounts and that serve to provide a store of amino acids and sulfur required during germination and seedling growth. Quality of seeds is driven by the total protein content present in the form of storage reserve in seeds. There are major four types of storage proteins known as-globulins (insoluble in water), albumins (soluble in water), prolamins (soluble in alcohol) and glutelins (soluble in dilute acid and alkaline medium). Globulins and albumins are the major storage seed proteins of legumes and oilseed crops whereas prolamins and glutelins are mostly found in cereal seeds. Functionally boron is crucial micronutrient for a considerable amount of agricultural yield. Seed reserves (proteins, carbohydrates, starch, lipids) of post harvested seeds are depended on the appropriate boron supply during cropping. Boron insufficiency in oilseed crops found to be an inhibitory factor for seed vigor and seed quality. So this chapter deals with the effect of boron deprivation on seed quality in terms of germination capacity and seed storage protein reserves in the post harvested seeds of soybean, mustard and linseed.

Keywords: boron, oilseed crops, storage proteins, seed germination, seed yield

1. Introduction

Boron is essential for appropriate reproductive blooming in crops. Reproductive growth is more sensitive to boron deficiency and failure in pollination, abscission of reproductive organ or falling of young fruits are typical symptoms of deficiency [1]. The boron requirement is much higher for reproductive growth than for vegetative

growth in most plant species. Boron increases flower production and retention, pollen tube elongation and germination and seed and fruit development. The reproductive growth especially flowering, fruit and seed set and seed yield is more effective even at moderate boron insufficiency than vegetative growth [2, 3]. Various reports in many crops exhibited that boron can be inadequate and have a notable impact on yield even when there are no vegetative symptoms of boron insufficiency and supply of boron is also adequate [4, 5]. Shedding of buds, flowers and developing fruits and seeds as well poor fruit/seed quality and poor seed viability are seen in crops grown under inadequate boron at the onset of reproductive blooming [6].

Male sterility and retarded microsporogenesis and pollen fertility in wheat due to poor translocation of boron from vegetative to reproductive parts is the main cause of poor grain yield [7]. Post fertilization development and seed maturation is also influenced by the boron nutrition. Poor germination and vigor of seeds was reported in low boron crops [8]. Increment in phenolic compounds and fall in oil content in seeds of *Sesamum* plants receiving inadequate boron nutrition was spotted by Sinha *et al.* [9]. Chatterjee and Nautiyal [10] reported that boron deprivation in sunflower caused morphological aberrations in seeds and bring down the seed content of non-reducing sugars, starch and oil, even at the commencement of anthesis.

The demand of boron nutrition at the time of flowering and seed set is much higher in many crops even when boron concentration in vegetative organs are in appropriate amount Increased abnormal seedlings and decreased germination rate during seed germination was observed in boron insufficient seeds [8, 11, 12]. Various workers have reported that there is an enhancement in fruit set and yield with boron foliar fortification [5, 13]. The same was observed during the reproductive stage of sunflower leads to increment in seed yield suggesting involvement of boron in reproductive biology [4] and also qualitative and quantitative improvement in strawberry fruits [14].

Dordas [15] mentioned the consequences of boron foliar fertilizers on pod development, pod set, seed set, seed yield and yield components such as pod number per inflorescence, seed number per pod, seed development, seed weight, and on seed quality in terms of seed germination and seed vigor and observed that the seed yield was enhanced approximatly 37% and seed germination and seed vigor improved upto 27% in alfalfa as compared to untreated control. They also noticed that the critical boron levels for alfalfa used for forage production is below that for seed production and boron foliar fertilizeration can enhance the seed yield and seed quality of alfalfa grown for seed production. Pandey and Gupta [16] reported improved seed yield and seed vigor in the boron insufficient black gram plants given foliar application of boron. They also reported improved seed quality in terms of storage seed proteins (albumin, globulin, glutenin and prolamin) and carbohydrates (sugars and starch) in black gram received foliar boron fertilizer. The reality of that boron implementation improved seed vigor specifies that seeds with adequate boron nourishment can germinate and produce seedlings with better ability to grow and resist any adverse environmental conditions. This is in agreement with the suggestion that seeds that were developed in plants with adequate nutrient supply show high germination percentage and also have high seed vigor [17].

The essentiality of boron suggests that there is a continuous requirement of it during the entire growth period of plants. There is a critical requirement of boron for reproductive development and seed quality which must be met at the onset of the reproductive phase. Thus boron deficiency is a major factor responsible for low seed yield and quality of oil yielding crops widely cultivated on boron deficient soils world over. The aim of the research work conducted is to put together the effect of boron on reproductive development, production, maturation and nutritive quality

of soybean, mustard and linseed seeds. In agriculture especial attention is required for achieving higher production and productivity levels in agricultural and horticultural crops which are essential for nutritional security. Thus the crops chosen for the work are the widely consumed soybean, mustard and linseed as they meet the daily cooking oil requirement in India. This chapter also suggests that there is an adequate requirement of boron nutrition to improve seed quality in terms of germination capacity and seed storage protein reserves in the post harvested seeds of soybean, mustard and linseed.

2. Experimental design and material and methods

Design of experiment is completely randomized and selection of plants was seasonal based during the work carried out in the laboratory. Plants were raised in the glass house under controlled conditions of light (light (PAR) ranged between 1050 to 1180 μmol m^{-2} s^{-1} at 12.00 noon), humidity (80–92%) and temperature (maximum and minimum temperature ranged between 37 and 43°C and 28 and 34°C respectively).

Sand culture: Soybean (*Glycine max* var. JS-335), mustard (*Brassica juncea* var. varuna) and linseed (*Linum usitatissimum* var. R-552) were grown in sand culture using the technique developed at the Long Ashton Research Station, Bristol, U.K. [18] and standardized for Indian conditions by Agarwala and Sharma [19]. The composition of nutrient solution used for growing the plants excluding B was: 4 mM KNO_3, 4 mM Ca $(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.1 mM Fe EDTA, 10 μM $MnSO_4$, 1 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.1 μM Na_2MoO_4, 0.1 mM NaCl, 0.1 μM $CoSO_4$ and 0.1 μM $NiSO_4$. Boron was supplied as H_3BO_3 at varying levels as sufficient amount (0.33 mg L^{-1} B supply) and insufficient (0.033 mg L^{-1} B supply) amount.

Seed germination: Seeds of all crops were first surface-sterilized with 5% (v/v) mercuric chloride solution and washed properly with deionized manesty still water (MSW) before germination. Sterile seeds were then sown in petridishes lined with three fold filter paper in double distilled water at 28°C and 85% relative humidity in seed germinator. The percentage germination of post harvested seeds of all crops was recorded after 48 h.

Seed protein: Seed protein was extracted by the method of Sommour [20]. After the harvesting of crops seeds were collected and seed coat was removed and seeds were ground in acetone. The extract was centrifuged 3–4 times at 11,500 xg for 10 min. Portions of air dried seed flour (200 mg) were extracted with water for albunins, 5% NaCl for globulins, 0.1 N NaOH for glutenins and 70% ethanol with 2 drops of mercaptoethanol for prolamines at room temperature. The proteins in the above extracts were estimated by the method of Lowry et al. [21]. The optical density of the reaction mixture was measured on spectrophotiometer at 750 nm. The readings were referred to a standard calibration curve prepared from crystalline bovine serum albumin.

3. Oilseed crops

Oilseed crops have been the backbone of agricultural economy of India from times immemorial. Today these crops are cultivated on about 26.67 million hectares, with total production of 30.06 million tonnes [22]. This area constitutes approximately one-tenth of the total cultivated area in India. On the oilseed map of the world, India occupies a prominent position, both in regard to acreage and

production. Oilseed crops are grown primarily for the oil contained in the seeds. The major world sources of edible seed oils are soybeans, sunflowers, rapeseed, cotton and peanuts. Seed oils from Flax (linseed) and castor beans are used for industrial purposes. Oilseed crops used for experimental work in the present study are:

Soybean: The soybean (U.S.) or soya bean (UK) (*Glycine max*) is a species of legume native to East Asia and originated in China. It is also known as a 'miracle crop' with over 40% protein and 20% oil. The plant is classed as an oilseed rather than a pulse. Soybean oil contains significantly greater amount of omega-6 fatty acids in the oil: 100 g of soybean oil contains 7 g of omega-3 fatty acids to 51 g of omega-6: a ratio of 1:7. Flaxseed, in comparison, has an omega-3: omega-6 ratio of 3:1. Soybeans also contain the isoflavones genistein and daidzein, types of phytoestrogen, that are considered by some dietitians and physicians to be useful in the prevention of cancer and by others to be carcinogenic and endocrine disruptive. Soy's content of isoflavones is as much as 3 mg g^{-1} dry weight. Isoflavones are polyphenol compounds, produced primarily by beans and other legumes, including peanuts and chickpeas [23]. The major unsaturated fatty acids in soybean oil triglycerides are 7% α-linolenic acid (C-18:3); 51% linoleic acid (C-18:2); and 23% oleic acid (C-18:1). It also contains the saturated fatty acids 4% stearic acid and 10% palmitic acid. The soybean seed storage proteins are classified in 8-conglycinin (7S) and glycinin (liS) proteins and multiple genes are responsible for their production [24]. The seed storage proteins of soybean are reported to be devoid of the S-containing essential amino acids, methionine and cysteine. Seed proteins consist of subunits dissimilar in amino acid profile i.e. 8-subunit of 7S protein. Approximately 60% l1S proteins consisting 3–4.5% sulfur amino-acid content and 40% 7S proteins consisting >1% sulfur amino-acid content are present in storage protein of soybean crops [25, 26].

Mustard: Rapeseed mustard (*Brassica*) contributes 32% of the total oilseed production in India, and it is the second largest indigenous oilseed crop. This species is of Asiatic origin and grows in southern regions of the Former Soviet Union, the Caucasus, Western and Eastern Siberia, the Far East, Central Asia. Generally it is also distributed in Middle Europe, Asia Minor, Iran, Afghanistan, India, Mongolia, China, Japan. *Brassica juncea* infests all spring crops including grain and tilled crops, and vegetable gardens. This plant is cultivated as an oil crop and for preparation of mustard powder in the south-east of the European part of Russia, in Ukraine, Belorussia and the North Caucasus. Seeds of *B. juncea* contain 25–30% fatty non-drying oil and glycoside sinigrine. Oilcake is used for preparation of mustard powder. Leaves are used for food in salads, they contain up to 150 mg of ascorbic acid. *B. juncea* is a good bee plant. Transgenic Indian mustard are also useful in phytoremediation of heavy metals and metalloids such as Cd, Cr, Cu, Mn, Zn, Pb, Hg, As, and Se [27, 28]. Indian mustard (*Brassica juncea*), are suitable target species for this strategy because of having a large biomass production, a relatively high trace element accumulation capacity [29], and can be genetically engineered [28]. Mustard oil is a healthy cooking medium because of low saturated fatty acids (8%), high monosaturated fatty acids (70%) and α-linolenic acid (10%) [30]. The mustard seeds are rich in lysine with considerable amounts of sulfur containing methionine and cysteine amino acids. Thus the mustard seed protein is the excellent source of human nutrition [31]. The most common seed storage proteins found in the cotyledons is cruciferin and napin. Cruciferin proteins are of 12S legumin-like globulin protein with mol wt 300-360 kDa and napins belongs to 2S prolamin-super family albumin with mol wt 12.7–20.3 kDa. These both proteins are differing in molecular forms, amino acid profiles as well as physico-chemical and biological properties.

Linseed: Flax also known as common flax or linseed (*Linum usitatissimum*) is a member of the family Linaceae. It is native to the region extending from the eastern Mediterranean to India and was probably first domesticated in the Fertile Crescent. Flax seeds contain high levels of lignans and omega-3 fatty acids. Lignans may benefit the heart; possess anti-cancer properties and studies performed on mice found reduced growth in specific types of tumors. Initial studies suggest that flaxseed taken in the diet may benefit individuals with certain types of breast and prostate cancers [32, 33]. Linseed oil is a rich source of linolenic acid (40–60%), an omega 3 fatty acid which has anti-inflammatory action in the treatment of arthritis. It also helps in lowering down the cholesterol level in mammals. Lignan present in oil has anti-carcinogenic effect [34]. Legumin- like proteins of mol wt 320 ± 20 and subunit mol wt of 55, 54.5,50,45,43 and 41 is the characteristic feature of majority of linseed seed proteins. These proteins contain non –covalently bound carbohydrates with pI- values 4.5 to 8. The 2- mercaptoethanol reduces the legumin-like protein polypeptides into acidic and basic subunits with mol wt 25–40 and mol wt 18–22 respectively. Albumin –like proteins contained major subunit with mol wt 25 and minor subunit with mol wt 11 [35]. Sammour et al. [35] also reported that metabolic or antimetabolic activitiy is not performed by both types of subunits and their storage functioning is found to be due to abundance in nitrogenous amino acids.

4. Boron

Boron is a member of the subgroup III of metalloids and has intermediate properties between metals and non-metals. The boron atom is small and has only three valencies and has a strong affinity for oxygen. Boron is present in soil solution in different forms- BO_2^-, $B_4O_7^-$, BO_3, $H_2BO_3^-$ and [B (OH) $_4$]. As an uncharged molecule, its permeability coefficient for transport across the lipid bilayer is several orders of magnitude higher than that of ions. The physical and chemical properties of boron and its complexes are unique and highly varied. Under physiological conditions and in the absence of interaction with bio-molecules, boron exists as boric acid ($B[OH]_3$) or borate anion (B[OH4]-). Boric acid is a very weak acid, with a pKa of 9.24 a cytoplasmic pH (pH 7.5), more than 98% of boron exists in the form of free $B(OH)_3$ and less than 2% exists as $B(OH)_4^-$ [36]. At pH values found in the apoplast (pH 5.5), greater than 99.95% of boron is in the form of $B(OH)_3$ and less than 0.05% is in the form of $B(OH)_4^-$. Boric acid and borate however can readily react with many kinds of biological molecules and under normal biological conditions available boron binding molecules exceed the concentration of free boron. An understanding of boron binding reactions is therefore central to an understanding of boron physiology [37].

Boron is widely distributed in lithosphere and hydrosphere, boron concentration ranging from 5 to 10 mg kg^{-1} in rocks [38], 3–30 μg kg^{-1} in rivers [39] and ~ 4.5 mg L^{-1} in ocean [40]. Boron is found mostly in the topsoil. Dry weather reduces moisture in the topsoil and boron uptake by the plant, causing boron deficiency. Even high rainfall areas witness leaching out of borosilicate from the soil, which leads to boron deficiency. Boron deficiency is the most widespread micronutrient deficiency in agricultural crops in world including India. The symptoms of boron deficiency are observed when boron content in soil comes down to 5 to 25 mg ha^{-1}. Aluminum hydroxide adsorbs large amounts of soluble boron, making the soil acidic and causing boron deficiency. Soils containing a high proportion of organic matter are less deficient in boron.

Some specific symptoms found in plants grown under inadequate boron nutrition are: 'Top sickness' of tobacco; 'Corky core' or 'internal cork' or 'drought spot' of

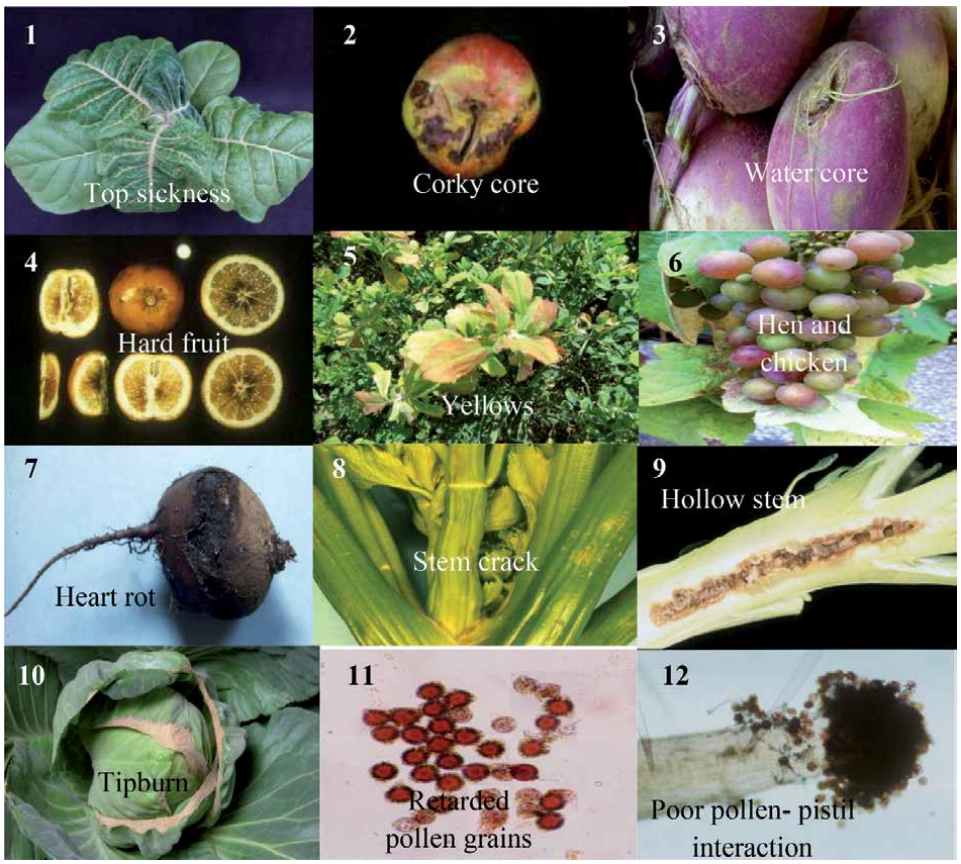

Figure 1.
Various symptoms in plants received inadequate boron nutrition (data source online except 11 and 12 these are original data from our experiment).

apple; 'Water core' of turnip'; 'Hard fruit' of Citrus; 'Yellows' of alfa alfa; 'Hen and chicken' of grapes; 'Heart rot' of sugarbeet; 'Stem crack' of celery; 'Hollow stem' of cauliflower and broccoli and 'Tipburn' of chinese cabbage ([41, 42]; **Figure 1**). Boron deficiency also creates an unfavorable situation for the pollen-stigma interaction and limits fertilization leading to poor reproductive yield ([42]; **Figure 1**). Boron takes part in various vital functioning of crops like translocation of sugars, metabolism of RNA, protein, indole acetic acid, phenols, ascorbate, osmotic and oxidative stress etc. [1, 43, 44].

5. Storage proteins

Seed yield is a complex trait as it is the product of several individual yield components such as number of inflorescences per plant, number of pods per inflorescence, number of pods per plant, number of seeds per inflorescence, seed weight per pods, seed weight per inflorescence and mean seed weight [45]. In oilseed crops oil is an essential component obtained from seeds. There are many factors that influence seed yield and seed quality such as genotype, agronomic techniques nutritional disorder and the environment. Seed quality is reflected in seedling density, seedling vigor, the competitiveness and uniformity of crop growth [46]. The quality of grains is basically depended on the storage proteins

present in their seeds. These proteins are mainly stored in seeds and came into existence at the time of seed development and acts as a nitrogen source during germination. [47] suggested that the polypeptide Polymorphic characteristics of storage protein is due to the existence of multigene families and this polymorphism can be seen within single genotypes as well as among genotypes of the same species.

The storage protein present in seeds ranging from ~10% (in cereals) to 40% (in certain legumes and oilseeds) of dry weight, are the fruitful source of dietary protein. Seeds comprised of one or more groups of proteins that are in abundance and acts as a store house for amino acids which can be use all along the seed germination and seedling growth. The total protein content present in seeds in the form of storage reserve determined the quality of seeds for various purposes [47]. Among several kinds of proteins four major types of storage proteins are known- globulins (insoluble in water), albumins (soluble in water), prolamins and glutelins, which are alcohol and alkali soluble respectively [48]. Globulins and albumins are the major storage seed proteins of legumes and oilseed crops whereas prolamins and glutelins are mostly found in cereal seeds. All four types of storage proteins are classified as simple globular proteins.

5.1 Albumins

Albumin proteins are mostly found in dicot seeds. These storage proteins are accumulated in the protein bodies of developing seeds and are serve as a source of sulfur-containing amino acids and carbon skeletons for providing nutrition to the growing seedlings and germination of the seeds. Despite of their physiological role, these small globular proteins in plants are also having an area of interest in the field of nutritional and clinical studies [49]. Agizzio *et al*. [50] have investigated that 2S albumins can also acts as defensive weapons for the protection against fungal invasion in plants. Arabidopsis and oilseed rape (family- Cruciferae) plants are most commonly used to study these proteins. In oilseed rape plants this protein is named as napins. Ericson et al. [51] suggested that the napins consist of two polypeptide chains with *M,* values of −9000 and 4000, linked together with interchain disulfide bonds. The synthesis of napins gave rise a single precursor proteins in which proteolytic cleavage occurred. Due to this cleavage the loss of a linker peptide and short peptides from both the N and C termini was reported [51, 52]. All the 2 s albumins are compact globular proteins with conserved cysteine residues inspite of differing in their subunit structure and synthesis.

5.2 Globulin

Globulin storage proteins are found to be stored in the embryo and outer aleurone layer of the endosperm [53]. These proteins having sedimentation coefficients, approximately 7 and are found to be quickly dissolve in dilute salt solution. Kriz and Wallace [53] examined that the protein bodies are the main storage site for 7S globulins. The most primitive cupin superfamily is the representative of globulin proteins. On the basis of their sedimentation coefficients, they are of 11S legumin and 7S vicilin types. Globulins perform various functions such as sucrose binding, desiccation, defense against microbes, hormone binding and oxidative stress etc. in plant and also have nutritional values in seeds [54]. The functioning of globulin proteins in seed development was investigated by Hye-Jung Lee *et al.* [55]. According to their findings, deficiency in globulin induces the reduction in the expression of other seed storage proteins (glutelins and prolamins) in dry seeds of a rice mutant (Glb-RNAi) as compared to wild type. They also suggested that the

globulin might have a crucial role in a transcriptional mechanism and in the de novo protein maturation process of storage proteins in the rice endosperm.

5.3 Prolamins

About 20% -30% seed protein is comprised of prolamins. Multigerne family of 34 gene copies having relative molecular weights –10, 13, and 16 kD encoding the prolamin proteins. Among them the 13 kD molecular weight gene family comprises the major group. Further, on the basis of abundance of cysteine residues the 13 kD prolamins are classified in class I, II, or III, [56, 57]. Prolamins are the main storage proteins in the endosperm of all cereal grains. These proteins are basically rich in proline and amide nitrogen which is derivative of glutamine. The prolamins contains variable molecular masses ranging from approx 10 000 to 100 000. Miflin et al. [58], on the basis of amino acid sequencing, classified the prolamins into three groups namely S-rich, S-poor, and high molecular weight (HMW) prolamins. Among them S-rich prolamins are found to be about 80 to 90% of the total prolamin fractions consisting of monomeric and polymeric components with intrachain and interchain disulfide bonds respectively. The most abundant prolamin group among rice storage proteins is 13 kD prolamins that is indigestible in nature (Hyun-Jung [59]). Kim et al. [59] have generated transgenic rice plants (13 kD pro-RNAi) consisting of RNAi that are constructing against 13 kD prolamins. They reported in their results that 28% increase in the level of lysine, and abnormal formation of PB-I (protein bodies) in the transgenic grains might be due to the reduction in 13 kD prolamins at the mRNA and protein levels.

5.4 Glutelins

Glutelins are the member of the class of prolamin proteins. The seed endosperm of the grass family is mostly enriched with glutelin proteins. Gluten is the main component of the glutelin protein. Zhao et al. [60] established that about 70–80% glutelins are present mostly in rice. They also reported that glutelins are homologous to 11-12S globulin proteins of leguminous family. Wakasa *et al.*, [61] explained that the pre-proglutelins are the initial precursor of glutelins and due to hydrophobic interactions in the lumen of the rough endoplasmic reticulum they form homotrimers and heterotrimers. [62] reported the 15 glutelin genes in the rice genome, and classified them on the basis of their amino acid sequences into four groups- Glu A, Glu B, Glu C, and Glu D. Glu A consists of three members Glu A1, Glu A 2 and Glu A 3 and Glu B have four members Glu B1, Glu B2 Glu B3 Glu B4. Takahashi *et al*., [63] demonstrated the localization pattern of five subtypes of the glutelin protein in rice grains with the help of glutelin-subtype specific antibodies. They reported that the localization of GluA was strongly in the outer region of the endosperm, including the subaleurone layer, and that of GluC was localized throughout the endosperm.

6. Impact of boron on seed germination and seed protein concentration

Post harvested seeds of all crops received boron inadequate nourishment were reduced in size and showed poor rate of germination (**Figure 2**). Seed germination was found to be retarded 37%, 36% and 43% in soyabean, mustard and linseed respectively. All the storage protein fractions- albumins, globulins, glutelins and prolamins were decreased in seeds of boron inadequate supplied plants as compared to plants supplied with appropriate amount of boron. In soyabean and mustard prolamins was found to be marked reduced as compared to other protein fractions

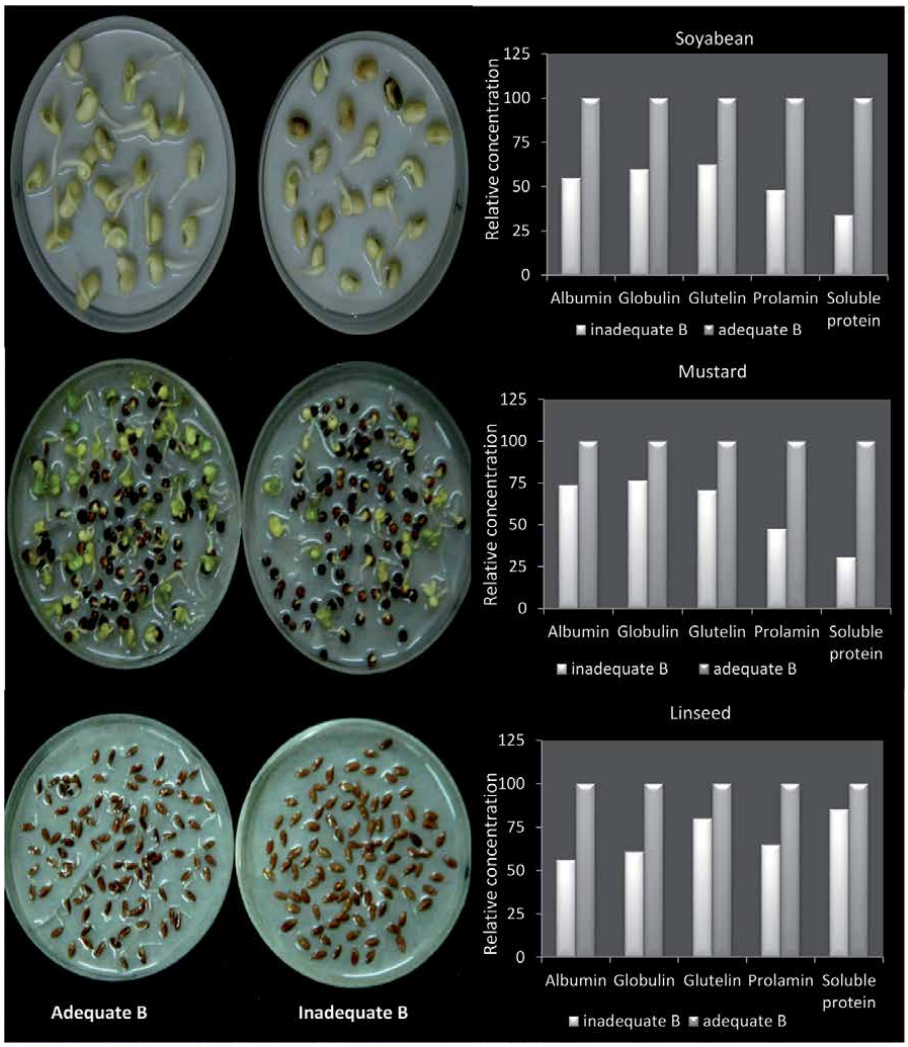

Figure 2.
Impact of boron on seed germination rate and seed storage protein concentration.

and globulins (in mustard) and glutelins (in soyabean) are least effective. In linseed seeds albumins were found to be decreased more than other fractions and glutelins were least decreased. In soyabean seeds decrease in prolamins and soluble proteins (≈52% and 66% respectively) were found to be more than albumins, globulins and glutelins (≈45%, 40% and 38% respectively). In mustard seeds also decrease in prolamins and soluble proteins (≈52% and 69% respectively) were found to be more than albumins, globulins and glutelins (≈26%, 23% and 29% respectively). In linseed seeds decrease in glutelins, prolamins and soluble proteins (≈20%, 35 and 14% respectively) were found to be less than albumins and globulins and (≈44% and 39% respectively) (**Figure 2**).

7. Conclusion

Inadequate boron nourishment may be a major factor responsible for low seed yield and quality of oil yielding crops widely cultivated on low boron soils world

over. Plants receiving inappropriate amount of boron nutrition showed decrease in all protein fractions. This might be due to increased activity of ribonuclease which disturbed the protein synthesis mechanism via influencing the RNA content of a cell. An increased ribonuclease activity in various crops under boron stressed condition was earlier observed by many workers [64, 65]. The observation made on the basis of results obtained in the present study suggested the role of boron in protein metabolism of seeds and that optimum concentration of boron is required for the appropriate synthesis of protein. Deformed seed structure and poor storage capacity for reserves might be the cause poor rate of seed germination. It is hoped that the information generated on the basis of this study will add to the information regarding the role of boron in seed protein reserve and quality improvement of seeds.

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Climate resilience and growing population are the two main global challenges that encourage the development of an affordable and sustainable source of vegetable protein to ensure future food security. Advanced scientific programs and agro-food developments should be proprietarily on-demand to face different stresses in order to maintain yield and quality of seed production. In this regard, legume crops are key sustainable alternatives for healthier diets while contributing to appropriate natural resource management.

Taken together, the 11 chapters in this book represent a generous addition to the progress in our understanding of climate-resilient legumes, hoping to contribute to the improvement of global food security in the future.

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