Chapter

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

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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

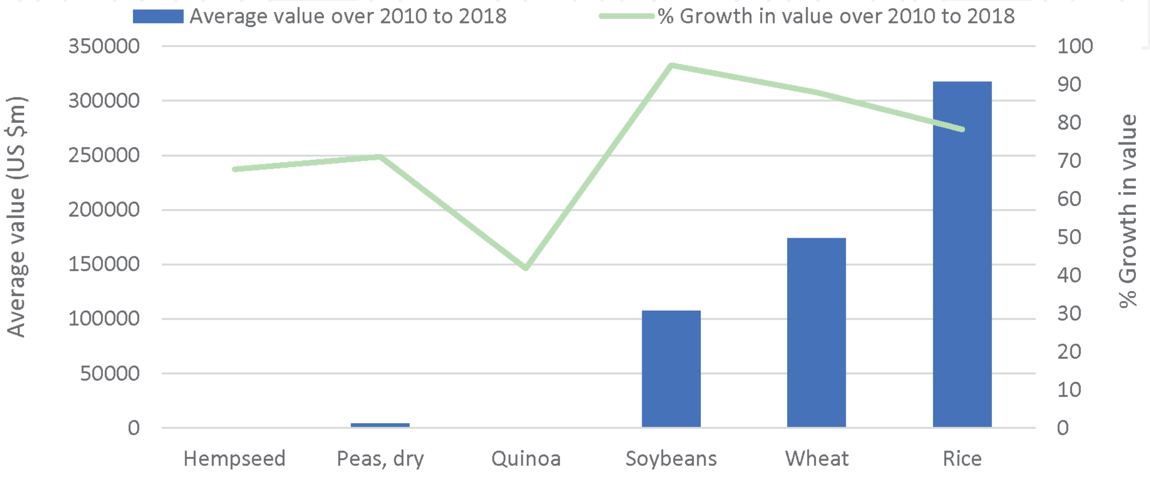

Figure 1.
Value of major grain commodities used as ingredients for producing plant-based protein foods over the last decade. Data adapted from FAOSTAT [11].

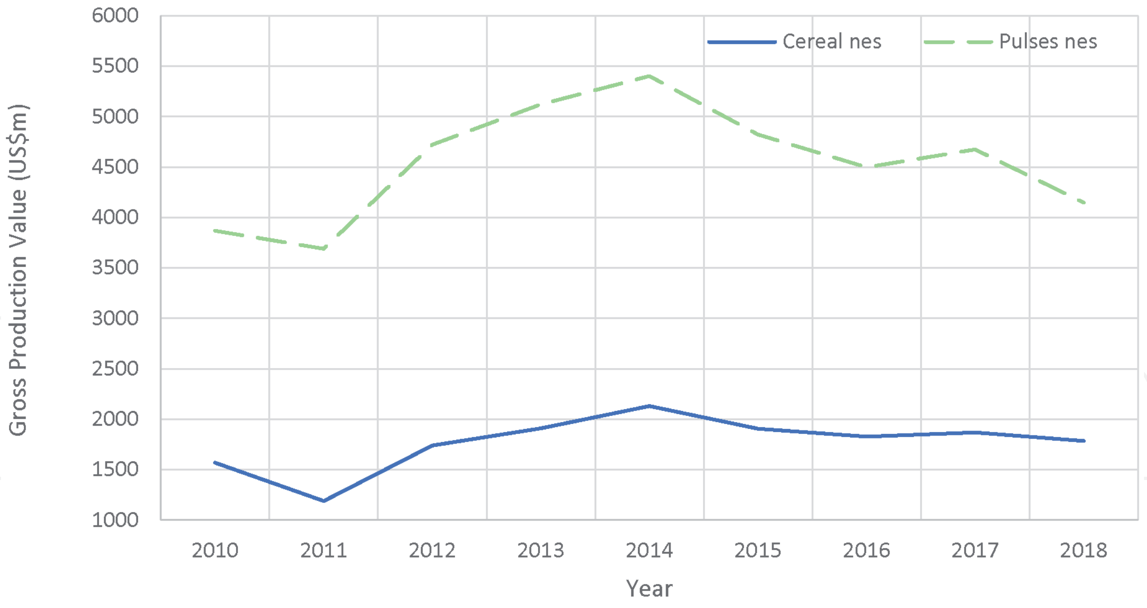

Figure 2.
Gross production value of cereals and pulses grain crops over the last decade. Data from FAOSTAT [11].

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,

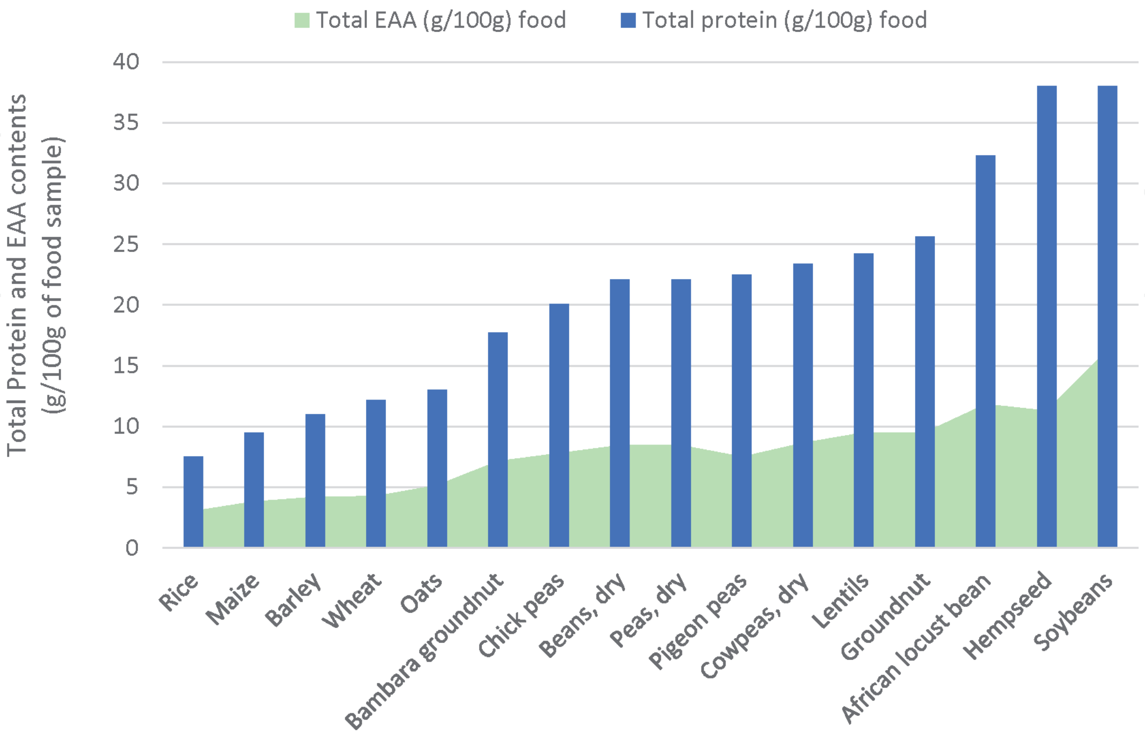

Figure 3.
Dietary protein and equivalent essential amino acids (EAA) of cereals and legume sources. Data from FAO [11].

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

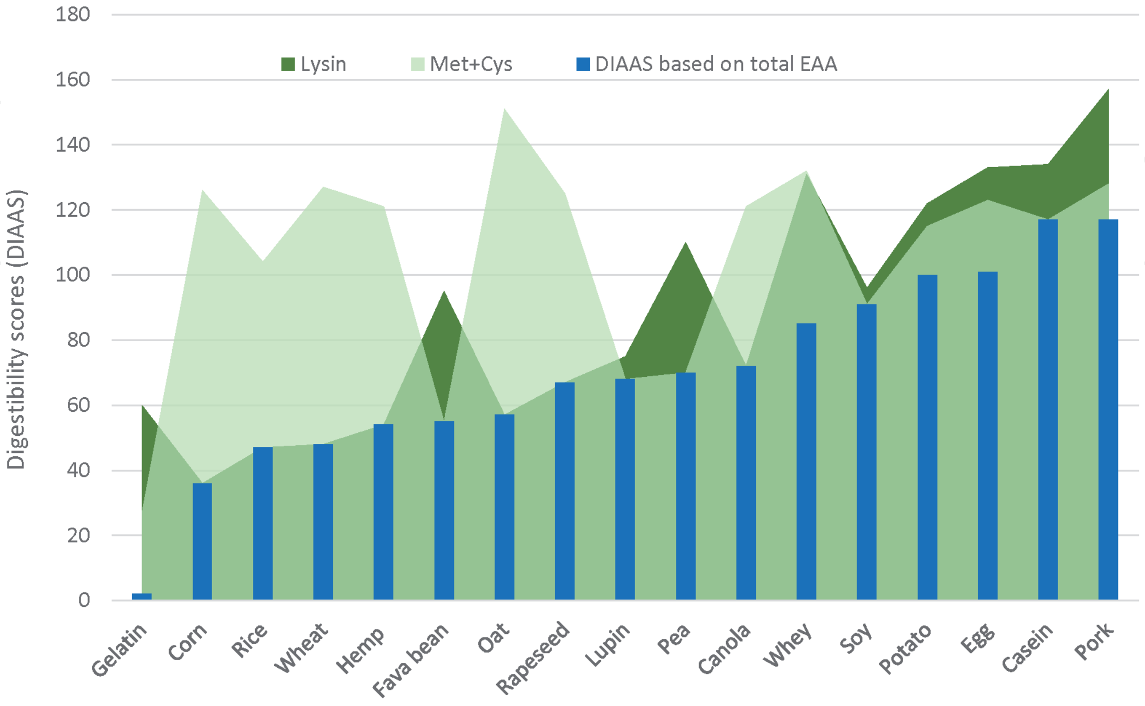

Figure 4.
Digestibility scores (DIAAS) of limiting EAAs (lysine and methionine+cysteine) and DIAAS of 17 dietary protein sources according to the 0.5-to 3-year-old reference pattern score. Data from Herreman et al. [34].

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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