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REVIEW

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Protein characteristics, amino acid profile, health benefits and methods of extraction and isolation of proteins from some pseudocereals—a review

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Abstract

Pseudocereals are the seeds or fruits of non-grass species that can be consumed similarly to cereals. Most commonly used pseudocereals are amaranth, quinoa, buckwheat, chia, album and wattleseeds. These underutilized pseudocereals are good source of essential amino acids, essential fatty acids, phenolic compounds, vitamins, flavonoids and minerals. Food and Agriculture Organization (FAO) has identified many plants as under-utilized, which can significantly contribute for improving nutrition and health, enhance food basket and livelihoods, future food security and sustainable development. They are reported to have anti-cancerous, cardio-protective, anti-inflammatory, hypocholesterolemic, anti-obesity and antioxidant properties. This review paper portray major pseudocereals with their amino acid composition, methods of extraction and isolation of proteins, effect of processing on the quality of protein and nutritional profile and various health benefits.

Keywords Pseudocereals, Amino acids, Quinoa, Protein extraction, Gluten free, Amaranth, Buckwheat

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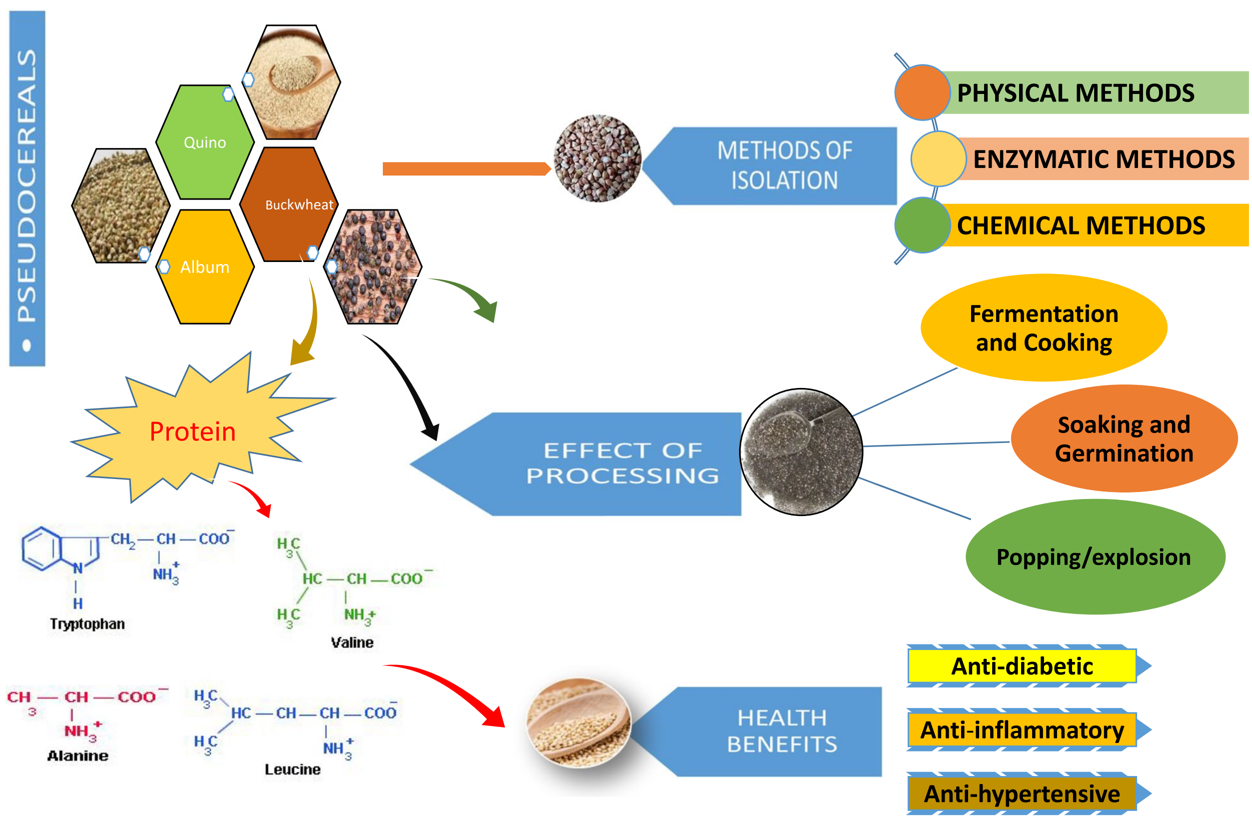
Graphical Abstract



Introduction

As the world's population grows and natural resources decline, there is a growing concern about the availability of sustainable healthy foods. Furthermore, majority of the population in developing nations experience severe protein malnutrition, hunger and various illnesses as a result of insufficient food availability and poor food quality (Schonfeldt & Hall 2012). Proteins derived from animals are of high quality, but they are often expensive (Spychaj et al. 2018). Plant proteins can supplement or even replace animal proteins as a source of essential amino acids. Healthy diet is critical in the prevention of numerous civilization-related disorders, including diabetes, cardiovascular problems, as well as obesity (Gramza-Michałowska 2020). Nutritional guidelines are issued by both state entities and Non-governmental organizations in order to safeguard public health, prevent the development of illnesses, and ameliorate associated symptoms. The development of novel functional foods is an appealing trend in the food processing sectors (Bigliardi & Galati 2013). Because of the inclusion of nutraceutical components, functional foods provide unique health advantages in addition to their nutritional features. Nutraceuticals are therapeutic nutritional components that have an essential role in sustaining well-being, improving health, regulating immunity, and preventing lifestyle illnesses (Ashraf et al. 2020). Pseudocereals are promising crops of future due to their high heritability that is advantageous for them to be adapted to different environments from tropical to temperate climatic conditions. Because they contain no gluten, they are often used in gluten-free products. Pseudocereals are abundant in protein, fibre, as well as micronutrients and they also possess bioactive and health-promoting properties. Nowadays the consumers are opting for healthier options for healthy life (Khairuddin & Lasekan 2021). Because of the growing population there is a scarcity in the food supply. The FAO has quoted that major concern is rising undernourishment rate of population, which increased from 19.6 per cent in 2014–2016 to 21.8 per cent in 2018–2020 (FAO 2021). The basic component which is important for building a healthy anatomy is protein. The importance and the functionality of the grains mainly depends on the quantity and the quality of the protein they provide (Poutanen et al. 2014). Proteins from vegetable source are naturally occurring, low in cost and are considered safe for consumption. Pseudocereals are basically underutilized crops. They are high in protein and gluten free. Pseudocereals such as amaranth, quinoa and buckwheat are packed with nutritional components (De Bock et al. 2021). Development and utilization of these pseudocereals as novel foods having throng of health benefits will boost the public health. Pseudocereals are highly nutritious and contains a huge range of compounds namely, phenolic acids, vitamins, flavonoids and fatty acids. These components helps in prevention from degenerative diseases (Yadav & Upasana 2022). Researches has shown that inclusion of pseudocereals in daily diet can help in combating diseases such as cancer, diabetes and cardio vascular diseases. Due to their considerable genetic diversity, pseudocereals are considered as potential crops for the future which allows them to easily adapt to a variety of habitats, spanning from tropical to moderate environmental conditions (Joshi et al. 2019). Although they are high in nutrients such as minerals, amino acids, protein etc., they are not much commercialized due to the non-availability of modern technologies and equipments as well as research gap. The most popular pseudocereals are amaranth (*Amaranthus* L. spp.), quinoa (*Chenopodium quinoa* Willd), and buckwheat (*Fagopyrum esculentum* Moench and *Fagopyrum tataricum* (L.) Gaertn) (Pirzadah & Malik 2020).

Amino acid composition and protein in pseudocereals

Amino acids plays an important role in the alignment of protein in order to fulfil certain functions such as, biocatalyst, carrier for vitamins and minerals. They also help in formation of structural proteins (Malik et al. 2021). Dietary proteins contains amino acids which are essential for performing daily activities. The most common method used for the determination of amino acid composition is still the conventional technique which is carried out in two stages; first is hydrolysis and second is using chromatographic technique.

Recognized pseudocereals

Quinoa

Quinoa *(Chenopodium quinoa)*, a pseudocereal belongs to the *Chenopodiaceae* family. It is a dicotyledonous, an autogamous and self-pollinating species. It is also gynomonoecious and contains both hermaphrodite and female flowers on the same plant (Singh et al. 2021). It possess a huge amount of female flowers which are about 3 mm to 4 mm in size. The seeds range from 1 mm to 2.5 mm. Depending on the variety of quinoa, the color of the seeds can be red, white, yellow or light brown. This plant has its origin in the Andean region. The cultivation of this particular crop goes back to 8,000 years (Vega-Gálvez et al. 2010). Adaptation to different climate conditions is the main benefit of this crop. The plant of quinoa is salt, cold as well as drought tolerant. It can also grow in higher altitude. Quinoa has been consumed by peoples, because of its balanced amino acids and high protein content. It has drawn more attention because of its nutritive value and ability to adapt to the different climate and soil (Navruz-Varli & Sanlier 2016). This grain contains a higher percentage of protein and the major protein fraction are albumin and globulins. Ponce-Garcia et al. (2021) reported that quinoa has an unique amino acid profile that balances essential amino acids. Due to quinoa's great nutritional value, particularly the balance of vital amino acids, there is currently a resurgence in interest globally. As a result, quinoa proteins are viewed as prospective dietary components since they may be added to other protein sources to boost their nutritional value. It contains almost 10–16% dietary fiber. Quinoa is a gluten-free pseudocereal that has also caught the interest of gluten-free producers (Opazo-Navarrete et al. 2018). Typically quinoa is used as seeds for making soups and often consumed as breakfast cereals. Quinoa flour is also used in making food products such as bread, pasta, cookies, biscuits, etc. (Suárez-Estrella et al. 2018). Sprouting of quinoa can be done in order to enhance the vitamin content and for elimination of anti-nutritional factors (Di Cairano et al. 2018). The sprouts of quinoa is used as salad. Leaves are consumed just as spinach. The plant of quinoa has gained importance in recent years, as researches has shown its potential to be used as nutraceuticals and functional foods. According to some researchers it can also be used for therapeutic purposes (Złotek et al. 2019). In the year 2013, Food and Agriculture Organization of the United Nations introduced the year as the International year for quinoa, in order to enhance production, preservation and consumption of this underutilized crop. Overall, quinoa has greater protein and fat levels and a less carbohydrate content when compared to other cereals such as wheat, maize etc. (Li & Zhu 2018). Quinoa seed has a mean protein level of 12–23%, based on variety, which is greater than conventional cereals although less than oilseed crops and legumes (Toapanta et al. 2016). Past researches on quinoa protein confirmed that the main proteins in quinoa are globulins which accounts for approximately 37% of the total protein and albumins account for about 35%.

Quinoa contains low concentration of prolamins (0.5–7.0% of total protein) (Dakhili et al. 2019). Protein content in quinoa has been found to be of high value because of the presence of essential amino acids in a balanced manner. Proteins plays a significant role in the functional properties as well as structural properties of quinoa, like solubility, gel network formation, emulsification and foaming characteristics (Elsohaimy et al. 2015). Quinoa has almost all essential amino acids as suggested for intake by the standard bodies like FAO and WHO (Bastidas et al. 2016). Cereals and pulses lack some essential amino acids namely lysine, methionine and threonine. Quinoa has a high concentration of lysine which mainly ranges between 2.4 g/100 g of protein to 7.8 g/100 g of protein. The methionine content in quinoa is approximately in the range of 0.3 g/100 g protein to 9.1 g/100 g protein. The threonine content ranges from 2.1 g/100 g protein to 8.9 g/100 g protein (Nowak et al. 2016). Quinoa's compositional analysis revealed that its proteins are primarily deposited in the embryonic tissue, which also contains lipid, fibre, and saponin, whereas the perisperm contains large amount of starch (Ando et al. 2002).

Amaranth

Pseudocereals have protein content which varies between 10 and 20%. When compared to cereals and other legumes, the nutrient composition of proteins in pseudocereals is likewise quite high. Except for *Amaranthus spinosus* L., which grows in India's tropical and subtropical areas, Amaranthus spp. are endemic to Central and South America (TömöSközi et al. 2011). The Food and Agriculture Organization of the United Nations does not officially monitor amaranth output. Amaranth was traditionally a staple diet of Central America, but consumption declined after European colonization. Important producers include various South American nations, India and Kenya (Aderibigbe et al. 2022). Amaranthus and its varieties are traditional crops with high nutritional and medicinal value, but their true capacity is yet to be realized. The nutritional and bioactive components found in these plants contribute significantly to their capacity to provide health benefits (Cornejo et al. 2019). Amaranth contains a significant amount of lysine, tryptophan, arginine as well as sulphur containing amino acids. Amaranth has twice the lysine amount of cereals such as wheat and three times the lysine concentration of maize (Martínez-Villaluenga et al. 2020). The amino acid composition of amaranth doesn't really correlate well enough with cereals as well as legumes, but has the exception of sulphur containing amino acids which are abundant in amaranth in comparison to legumes. According to numerous publications, amaranth protein fractions contains albumin, globulin, prolamin and glutelin which are approximately 65%, 17%, 11% and 7% respectively. The amount of distinct fractions in the separated protein, as well as its functional and nutritional qualities are dependent on the extraction process employed (López et al. 2018). The principal storage proteins present in amaranth are albumins and globulins. Amaranth 11S, globulin P as well as 7S globulin are among the most important globulins. Albumins are high in sulphur containing amino acids as well as lysine and valine, whereas globulins are high in valine, leucine, glutelin, histidine etc., but still low in lysine. Because of the presence of different essential amino acids, the 11S fraction is greater than the other fractions (Tandang-Silvas et al. 2012). Amaranth provides additional essential amino acids, like lysine which acts as limiting amino acid in cereals. Amaranth also functions as a gluten-free pseudocereal, making it an effective gluten-free diet choice. The nutritional characteristics of amaranth have drawn the attention of researchers investigating the utilization of amaranth as a functional component. Earlier studies has shown that there is a significant difference in nutritious attributes amongst amaranth species, which includes amino acid composition, fatty acid composition and mineral content (Kachiguma et al. 2015).

Buckwheat

Buckwheat is a dicotyledonous crop that belongs to the *Polygonaceae* family. It is grown and modified to survive in extreme climate conditions in marginal areas. It is an annual herbaceous plant native to China as well as Central Asia. Buckwheat protein contains a diverse range of amino acids. It seems to be the only pseudocereal which contains rutin (Gondola & Papp 2010). Buckwheat peptides have antibacterial, anti-diabetic, as well as anti-aging properties. Buckwheat peptides as well as proteins revealed similar bio-functions to other peptides (Jin et al. 2022). It offers a wide range of possible dietary uses. Antioxidant, hypoglycemia, anti-tumorous and anti-hypertensive characteristics are among the health benefits (Zhu 2016a). Buckwheat is used to make a variety of edible products such as pasta, noodles, vinegar and bakery products (Cai et al. 2016).

Because protein is a significant constituent of buckwheat, the protein qualities can influence the nutritional properties and palatability of these products. Buckwheat contains a huge range of proteins which are of high quality. These high quality proteins have specific function to perform in the human body. Buckwheat proteins are significantly high in lysine, aspartic acid and arginine and have lower levels of glumatic acid as well as proline than cereal protein content. (Shukla et al. 2018). Protein in buckwheat is gluten-free however it contains allergens. Buckwheat proteins have a sturdy additional impact with the other proteins to enhance nutritional as well as amino acid balance. With special bioactivities such as cholesterol reducing effects and improving constipation, antihypertensive effects as well as obesity situations by acting similarly to dietary fibre and interfering with in-vivo metabolisms (Pihlanto et al. 2017). Aspartic acid (1301 mg/100 g of dry matter) and arginine (1496 mg/100 g of dry matter) are present in the buckwheat proteins. Glutamic acid and proline are present in less amount in buckwheat. Buckwheat contains high amount of lysine (624 mg/100 g of dry matter), which is a limiting amino acid in cereals (Satheesh & Fanta 2018). Due to this property of buckwheat it is considered as a healthier option when compared to other cereals. Prolamin, globulin, glutelin, albumin are present in the different varieties of buckwheat such as common (*Fagopyrum esculentum*) and tartary *(Fagopyrum tataricum*) (Ahmed et al. 2014).

Others

Chia seeds

Chia seeds (*Salvia hispanica* L.) also known as chia, belongs to Lamiaceae family and is an annual plant which grows in dry regions or semiarid climate zones. It has a high oil content and this oilseed is native to Mesoamerica (Sandoval-Oliveros & Paredes-López 2013). This pseudocereal has been grown in Mexico for centuries, and the recent studies have revealed that these seeds have a high potential for commercialization and their utilization for the human beings may have tremendous health benefits. Composition of these seeds appears to be very appealing, with high levels of antioxidants namely phenolic compounds such as chlorogenic as well as caffeic acids, kaempferol and quercetin. It also possesses high amount of dietary fiber approximately 30% (Alencar & de Carvalho Oliveira 2019). Chia seeds have higher protein content than most traditionally used grains. They contain about 19–23% of protein that is higher in amount when compared to cereals. Chia is not just a nutrient-dense food, but it also has the ability to be used as a functional food ingredient, such as a thickener. Its mucilage has been used to develop edible coatings and films (Muñoz et al. 2013).

The primary proteins in chia seeds are storage proteins, accounting for approximately 60–80%. Their evaluation is difficult by polypeptide heterogeneity and varying solubility behaviour. According to few research findings, edible seeds consists of mainly two kinds of storage proteins (major ones) that varies in size (Olivos-Lugo et al. 2010). The first group contains proteins with sedimentation coefficients about 11S, known as legumin like and 11S globulins. The second category proteins with sedimentation coefficients about 7S, also known as vicilin like and 7S globulins. Also there is a minor proportion of some other kind of protein known as 2S like proteins (Coates 2011). All amino acids, mainly the essential ones which are needed for human nutrition are found in chia seeds. Amid all amino acids, glutamine has the highest proportion, while histidine has the lowest. Chia seeds have a chemical score of 100% for sulphur amino acids and visibility for the leftover essential amino acids varies from 52%-76%. The essential amino acid content of seed flour ranges from 66%-100% (Gómez-Favela et al. 2017).

Album

Album grains are highly nutritious. They also provide nutrition which contains specified amino acids higher in quality that are deficient in most of our staple grains. *Chenopodium album Linn.* (Chenopodiaceae), is a Western Asian native plant. In India, it is represented by about 21 species, of which some are cultivated for an end use as vegetable and a few for the grains obtained from the plant (Mandák et al. 2012). Phytochemicals such as flavonoid, polyphenol, isoflavonoids and others have incited considerable interest due to its beneficial contribution in the maintenance of health, particularly a substantial decrease in cancer threat. They have also been discovered to be powerful antioxidants, capable of preventing or halting the oxidation process, a free radical chain response. *C. album* contains seven free phenolic acids such as gallic acid, protocatechuric aldehyde, caffeic acid, vanillin etc. Protocatechuric acids and gallic acid are discovered in the fruit part of album, whereas vanillin and m-coumaric acid are found in the leaves (Laghari et al. 2011). Vanillic and syringic acids were discovered in fruits as well as in leaves. *C. album* leaves have a high percentage of amino acids (essential) like lysine, and isoleucine as well as leucine, substantial quantities of micronutrients such as Vitamins A that is 11,000 IU/ 100 g and calcium 80 mg /100 g (Poonia & Upadhayay 2015). The lysine content in the seeds is approximately 8.07 g Kg^{-1} and threonine and leucine content are 5.32 g kg^{-1} and 7.58 g Kg^{-1}, respectively (Gęsiński & Nowak 2011).

C. album is a nutrient dense and edible wild weed which is native to India. Its green matter is a rich source of protein, which is yet another reason to cultivate these species. The overall amount of amino acids (exogenous) in green matter is relatively low, but this is due to reduced arginine content which is approximately 44%. With the exception of lysine as well as leucine, the amount of this amino acid in seeds is more than twice than that of other exogenous amino acids (Haber et al. 2017).

Wattleseeds

The genus *Acacia*, commonly known as wattle, belongs to the family *Fabaceae* and it comprises of a large group of woody species generally shrubs. Wattleseeds *(Acacia* spp.*)* is a well-known staple food popular among the indigenous communities in Australia. *Acacia* plants have been frequently used to treat diseases, such as throat infection, fever, haemoptysis, diarrhea and leucorrhoea. Shelat et al. (2019) studied the essential and non-essential amino acids of four distinct species of wattleseeds and the study revealed that all four had around 13–15 mol % dry weight glutamic acid, accompanied by approximately 9–11.5 mol % dry weight aspartic acid, 8.0–8.6 mol % dry weight leucine, 6.8–7.4 mol % dry weight lysine and approximately 7 mol % dry weight serine, arginine as well as alanine. Furthermore, methionine was found to be the limiting amino acid present in all four seeds, which is consistent with other seed species. Due to the high protein content and good nutritional profile of wattleseeds it could be a good option for inclusion in human diet and in various food formulations.

Methods of extraction and isolation of proteins

Grains are utilized in many food formulations that uses protein isolates as their primary ingredient, such as texturized vegetable protein and fitness supplement. Mainly alkalescent conditions are utilized when it comes to extraction of proteins from pseudocereals. The extraction and isolation of proteins from pseudocereals is becoming an increasingly relevant topic. In order to isolate proteins from the pseudocereals there are mainly three conventional methods namely (1) Physical methods, (2) Enzymatic methods, (3) Chemical methods (Malik et al. 2021). The yield of the extraction can be enhanced either by elevating the solubility of protein or breaking down the matrix which contains proteins. Changes in structure as well as composition, along with isomerization, bonding and degradation, often have techno-functional and physiological process repercussions (Deleu et al. 2019).

Physical methods

Physical approaches are employed because they are less difficult to modify and more cost effective than other protein separation techniques. Physical techniques are preferable when compared to enzymatic or chemical processes in food processing because they create less changes within the food (Fabian & Ju 2011). Physical actions that disturb cells boosts protein extraction. Milling, freeze thaw, mixing (high speed), blending and homogenization are some physical techniques utilized for extraction of protein. Shear pressure created during mixing as well as in homogenization is responsible for cell disruption. Sonication and chemical bonding will cause cell walls to break due to the consequent elevated temperature and shock waves, which produce cavitational bursting of the bubbles subjected to ultrasound power (Cravotto et al. 2013). Physical methods can produce items with higher convenience and safety, but the output of the desired properties is substantially lower. They are time consuming and resource intensive than other techniques of segregation, but they provide more monetary recovery to the approach. They are a viable substitute for wet extrication when utilized to produce the requisite proteinaceous mass, allowing us to acquire protein-embellished fragments with natural properties, which can be done only by dry fractionation due to their high energy efficiency (Schutyser & Van der Goot 2011). These traditional dry fractionation methods are divided into two steps. First one is the segregation of starch molecules from tiny protein rich substances through milling amid successive grouping. In the second one, the constituents are isolated by turbo-segregation or electrostatic segregation based on physicochemical differences. A substitute approach for constituent generation is conceivable via two-step dry fractionation, which avoids the inclusion of water while maintaining component activity (Laguna et al. 2018).

Chemical methods

Because of the underlying advantages it provides, the utilization of chemicals to isolate proteins from pseudocereals is one of the most extensively employed approaches. Aside from obtaining proteinaceous substances in pure state, the technology is also time efficient. When it comes to superior quality protein sources, only few pseudocereal meet the nutritional standards, and quinoa is one of them (Rollán et al. 2019). Proteins might well be extracted from quinoa by immersing quinoa flour (defatted) in deionized water with an alkaline medium. The solution was agitated and centrifuged to generate supernatants, which were then acidified as well as centrifuged once more to generate pellets and then resuspended into deionized water (Tian et al. 2020). Proteins with a purity of 90–93% were produced by high pH values, whereas proteinaceous substances with a purity of 82–88% were produced by low pH values. The results revealed that processing temperature had a greater influence on the quinoa protein components than the pH extraction (Ruiz et al. 2016). Because of the reduced fat level and higher protein content, the chemical approach proved more cost effective than that of the dry fractionation method for protein extraction. For such membrane aided salting out procedure, an ultra-centrifugal mill has been used to grind and sieve wattleseed, which was then stirred in tris–HCl buffer for the purpose of extraction. The resultant suspension was collected and then centrifuged to yield supernatant, which was then fractionated with increasing amounts of ammonium sulphate. The precipitates were dialyzed three times using tris–HCl buffer. Finally, the extracts were freeze dried and stored for further use. These findings revealed that secondary structures of proteinaceous fractions of wattleseeds are stable even at high temperatures, and especially at pH values that differed significantly (Agboola & Aluko 2009). Because of the high interaction of various components with each other, removing proteins from some pseudocereals is difficult and necessitates an entirely new technique. However one approach involved adding quinoa powder to inverse micelle solution and then placing it in a conical container. Extraction was carried out using a water bath oscillator, followed by centrifugation. After that, the system was separated into two layers, and the protein concentration was estimated. Temperature difference, narrow size distribution, and higher inverse micelle concentration were shown to be beneficial to the internal diffusion process. Diffusion of protein was indeed the previous phase of mass transfer throughout the AOT/isooctane extraction technique (Wu et al. 2018).

Enzymatic techniques

Enzymes have the capabilities to meet those needs whenever we seek for strategies to create a protein isolate of high quality as well as with selective customization of the constituents. Possibilities for segregating proteins as well as starch from amaranth flour were investigated as researchers designed techniques based on enzymatic approaches. Enzymatic disintegration of polysaccharides was employed in the directing process to liquify starch via hydrolyzing into glucose (soluble) and supplementing the solid substrate with vegetable protein (Mokrejs et al. 2011). A research was done by Elsohaimy et al. (2015) which was focused on development of the optimum conditions for the making of quinoa protein isolates. The functional and nutritional aspects of the protein isolate was determined in order to find out its potential usage in food industry. Quinoa protein isolate was produced by solubilizing protein at an alkaline pH, succeeded by condensation at an acidic pH of 4.5. In all extraction pHs, SDS-PAGE revealed protein bands of 55 KDa relating to globulin as well as 31–33 KDa relating to chenoprotein. Quinoa protein contained adequate levels of essential amino acids, besides tryptophan and a larger proportion of lysine that is 17.13%. The majority of buckwheat proteins were extracted using a solubilization and precipitation technique. The process starts with grinding the buckwheat into the flour. In order to solubilize the proteins, the flour was subsequently dispersed in a solution (alkaline) with a pH between 7.5 and 8.5. Isoelectric precipitation at a pH of 4.5 was used to collect the proteins. Before drying, the protein precipitate was rinsed to achieve a neutral pH (Haros & Wronkowska 2017). In other scenarios, salt solutions were used in the solubilization procedure (Sung et al. 2014). Amylases, particularly α-amylase as well as amyloglucosidase, were utilized to extract the protein fractions from buckwheat flour (Chen et al. 2019). The purity of extracted proteins ranged from 63 to 89% (Zhang et al. 2017).

Effect of processing on the quality of protein and nutritional profile

Food processing, in general, is intended to boost the nutritional content of food commodities by making natural components more easily available for process of digestion. To guarantee appropriate energy and nutrient extraction in plant-based foodstuffs, starch gelatinization, breakdown of cell walls and deactivation of harmful substances like toxins and anti-nutritional substances must be performed (Nikmaram et al. 2017). Furthermore, such seeds must be treated before they are consumed by humans. For contrast, multiple investigations show that processing procedures like boiling, fermentation, germination and malting enhances digestion and availability of micronutrients as well as consumer acceptability, flavor, consistency and taste. Some of the processes that effect the nutritional quality are mentioned below.

Cooking

Cooking can change the physicochemical characteristics of food, damage its nutritious components, and leads to protein oxidation, tryptophan breakdown, as well as protein carboxylation (Soladoye et al. 2015). In an experiment conducted by (Thakur et al. 2019) it was found that there was substantial increase in iron, zinc as well as calcium availability after roasting or boiling quinoa as well as amaranth grains. Thermal processing significantly reduced the overall phenolic content, antioxidant properties and flavonoids content in Tartary buckwheat flour. Motta et al. (2019) studied that during boiling and steaming of amaranth, the quantity of seven amino acids grew significantly compared to raw, which are alanine, lysine, serine, aspartic acid, threonine, leucine and valine. Buckwheat, in contrast, had a substantial drop in the quantity of six amino acids, particularly arginine, cysteine, methionine, histidine, tyrosine and threonine. Only sulphur containing amino acids showed significant alterations in quinoa, a reduction in cysteine and methionine compared to raw.

Popping

Amare et al. (2016) investigated the influence of popping as well as fermentation on the protein content of three distinct amaranth grain varieties particularly grown in Ethiopia. Amaranth seeds have more methionine, lysine and cysteine content than that of other legumes and cereals. Overall essential amino acid content (except tryptophan) was found to be about 43%—49%, greater than the reference pattern given by WHO that is 31%. Protein digestibility in-vitro reduced by 8.3%—17.1% after popping and by 4.8—7.5% after fermentation. Chemical components like fat, ash, acid detergent fiber and neutral detergent fiber concentration increased by 12%, 10%, 15% and 67% in amaranth, respectively (Amare et al. 2016). Amaranth protein digestibility (in-vitro) decreased by 8.3% to 17.1%. After baking, total phenolic content as well as antioxidant properties in red as well as in yellow quinoa seeds increased (Yael et al. 2012).

Fermentation

Pongrac et al. (2016) investigated the influence of fermentation on the quantity of phenolic compounds and antioxidants like isomers of tocopherol, ascorbic acid, as well as antioxidant properties in quinoa seeds. Fermentation significantly reduced the quantity of both tocopherol as well as ascorbic acid. During fermentation, antioxidant properties and phenolic constituents increased significantly. As a result, fermentation emerged as the most effective method for enhancing quinoa's bioactive capabilities. Amare et al. (2016) examined the impact of fermentation on the nutrient content of Ethiopian Amaranthus caudatus seed cultivars. Three cultivars were evaluated for chemical properties before as well as after fermentation processing. Fermentation increased the protein, fat, but also ash levels by 3%, 22% and 14%, respectively. Gobbetti et al. (2020) investigated the technical, nutritional and functional properties of several legumes and pseudocereals and discovered that sourdough fermentation was the most suited approach for utilizing these properties with widespread consumer acceptability. Ugural and Akyol (2022) investigated the nutraceutical possibilities of pseudocereals and found that their nutritional properties may be improved using a variety of processing techniques like sprouting and fermentation. These, through their prebiotic actions, can function as a foundation for the proliferation of probiotic microbes, particularly *lactobacillus* strains, and aid in the synthesis of short-chain fatty acids. As a result, they can serve as suitable sources for symbiotic formulations for those suffering from gluten sensitivity, lactose intolerance, overweight as well as inflammation-mediated chronic illnesses.

Soaking and germination

Hao et al. (2016) discovered that pre-germination treatment with mildly acidic electrolyzed water promoted the development of GABA that is γ-aminobutyric acid and rutin in buckwheat. The rutin content as well as GABA concentrations were 739.9 mg/100 g and 143.20 mg/100 g, respectively. Carciochi et al. (2016) investigated the influence of germination time mostly on content of antioxidants like phenolic content, antioxidant activity, tocopherol isomers as well as ascorbic acid in quinoa. When compared to raw seeds, the total amount of tocopherol as well as ascorbic acid considerably enhanced after 72 h of germination. During the germination phase, antioxidant properties as well as total phenolic compounds increased significantly. Kaur et al. (2016) investigated the total polyphenolic composition, vitamin C content and antioxidant properties of raw and commercially treated quinoa. The authors discovered that domestically processed seeds had greater levels of bioactive substances like total phenolic content, total flavonoid content as well as antioxidant properties than untreated as well as mechanically treated seeds.

Health benefits of different pseudocereals

Despite the fact that multiple studies have shown significant biological activity and various health-promoting qualities of pseudocereals for quite some time, a dramatic increase in interest in commercial uses of the pseudocereals has only lately been noted. For any food, it is crucial to consider not just the chemical properties of its commodities or the availability of nutrients in certain quantities, but also their physiological qualities such as bioavailability and nutrient retention after processing within the final product. Various health benefits of pseudocereals has been discussed in Table 1. According to a new study, intake of chia might completely or partially prevent hepatitis and lower cholesterol levels in the test rats. The hypolipidemic as well as hepatoprotective properties of chia are attributed to the high concentrations of phenolic compounds as well as omega-3 fatty acids (alpha-linolenic acid) (Fernández Martnez et al. 2019). Chicco et al. (2009) conducted an experiment that demonstrated the beneficial effect of chia seeds on the plasma lipid profile. In the study conducted on rats when it was compared to the control, rats which were fed with a diet rich in sucrose and incorporated 2.6% chia seeds had reduced blood levels of triglycerides, fatty acids and total cholesterol. There were no changes in blood glucose concentrations detected in trial. Furthermore, rats who consumed chia seeds had lower levels of visceral fat. Sierra et al. (2015) conducted an experiment to investigate the impacts of chia seed oil utilization on the blood lipid profile. They discovered that feeding 10% chia seed oil resulted in lower total cholesterol, Triglycerides and High Density Lipoproteins levels, simultaneously Low Density Lipoproteins levels improved. Nevertheless, it should be noted that statistically significant alterations were only observed for TG. It's been shown that sprouted chia seeds have higher protein quality, as evaluated by the protein efficiency ratio. Using

Table 1 Health benefits of pseudocereals and their key mechanism

Pseudocereals	Action mechanism	Doses	Models used	Key findings	References
Amaranth	Anti-diabetic	Amaranth protein hydrolyzates (25 animals) Duration: 4 weeks (chronic cases)	Streptozotocin induced CD1 mice	Major improvement of glucose tolerance Increase in plasma insulin in acute and chronic cases	Soriano-Santos et al. (2015)
	Anti-obesity	Control vs. amaranth protein diet in diet induced obesity (48 animals) Duration: 8 weeks	C57BL/6 mice	Increase caecal crypt depth and calceiform cells number Reduction in abundance of *Lachnospiracea* and *Ruminococcaceae* induced by high fat diet	Olguín-Calderón et al. (2019)
	Cardio-protective	Casein vs. amaranth protein (22 animals) Duration: 2 weeks	Wistar rats	Significant induction of clotting tests, activated partial thromboplastin time and thrombin time	Sabbione et al. (2016c)
	Hypertension	Control vs amaranth protein diet incholesterol-enriched diets (42 animals) Duration: 4 weeks	Wistar rats	Decreases of plasma total cholesterol and TG Increase of faecal cholesterol excretion	Lado et al. (2015)
Buckwheat	Antidiabetic	Buckwheat vs rice crackers Duration: one week Control vs. tartary buckwheat (150 g/day) Duration:4 weeks	Diabetic patients (Acute or chronic) 165 Diabetic Individuals	Reduction of level of serum glucose Decrease of fast insulin, total cholesterol, and LDL cholesterol	Wu et al. (2018); Stringer et al. (2013) Qiu et al. (2016)
	Antiobesity	Tartary buckwheat flour(50%) Duration: 8 weeks	144 adult subjects	Inhibits inflammation of adipose tissue Significantly decreased body weight and body fat percentage	Nishimura et al. (2016)
	Hypolipidemic	Control, and high fat diet with casein vs tartary buckwheat protein (27animals) Duration:6 weeks	C57BL/6 Mice	Decrease in the level of TC and TG Changes in post prandial plasma GLP-1, GIP, and pancreatic polypeptide after consumption of buckwheat	Chen et al. (2019) Zhou et al. (2018)
Quinoa	Antidiabetic	Control vs. quinoa seeds in high-fructose diet (24 rats) Duration:5 weeks	Wister rats	Blood glucose reduction	Pasko et al. (2010)
	Cardiovascular disease	Bread enriched with quinoa vs. refined wheat bread (37 individuals Duration:8 weeks	Overweight males	Modification of glucose response Minimal effects on other cardiovascular risk biomarkers (LDL-cholesterol)	Li et al. (2018b)
	Anticancer	25 g of quinoa flakes daily Duration: 4 weeks	34 women	Decreased interleukin-6, which is a marker of inflammation Tumor reduction	De Carvalho et al. (2014)

Table 1 (continued)

Pseudocereals	Action mechanism	Doses	Models used	Key findings	References
Chia seeds	Anti-inflammatory	Control vs.10% chia seed oil Duration: 35 days	40 BALB/c mice (male and female)	Decreased the estrogen receptor (ER) α expression, a recognized breast cancer promotor	Vara-Messler et al. (2017)
	Antioxidant property	Milled chia seeds (25 g) per day Duration: 07 weeks	Post-menopausal women(10 subjects)	Significant increase in serum ALA and EPA concentrations Helps in modulation of fatty acid composition of membrane	Jin et al. (2012)

updated recommendations for human health as well as food safety, Coelho et al. (2018) discovered that protein hydrolysates (chia by-products) may be used as hypocholesterolemic substances in functional foods as well as nutraceuticals. Another experiment was conducted to examine the effects of chia seeds on the plasma triglyceride levels of healthy people with controlled levels of lipid impairment. The findings showed that the chia can indeed be employed as a new, safe alternative medicine with therapeutic importance in the prevention of primary Cardiovascular diseases because it regulates bad cholesterol (Tenore et al. 2018). As demonstrated by Orona-Tamayo et al. (2015) the albumin and globulin portions revealed strong antiradical action against 2, 2-diphenylpicrylhydrazyl and prolamin, and also globulin's capacity to chelate ferrous ions. Several studies have shown that quinoa products offer distinct health benefits. Quinoa has been demonstrated to decrease cholesterol in studies conducted by (De Carvalho et al. 2014). In the experiment a 4-week prospective and double-blind randomized trial on overweight women found that consuming 25 g of quinoa flakes significantly lowered blood triglycerides, low density lipoprotein and total cholesterol. Quinoa has also been shown to have antioxidant properties in rats fed a high-fructose diet to produce oxidative metabolic stress (Pasko et al. 2010). According to Alghamdi (2018), quinoa seed powder was added to the diet of male rats to test if it might lower their cholesterol levels and prevent hypercholesterolemia. According to this in-vivo investigation, a meal enriched with quinoa seed powder increased feed consumption and muscle growth while lowering lipid profiles and organ function risk. Accordingly, quinoa seed powder can lessen the negative effects of hypercholesterolemia. Additionally, quinoa yoghurt's ability to decrease cholesterol was recently proved in a research (Obaroakpo et al. 2020). Omary et al. (2012) claimed that germinating pseudocereals have higher levels of nutrients, minerals, polyphenols, as well as antioxidant properties while having lower levels of anti-nutritional factors. As a result, germinated gluten-free pseudocereals have always had the potential to be employed as natural fortification as well as enrichment in gluten-free diets. Their incorporation, however, may impact flavor and texture of the product (Mäkinen et al. 2013), which requires additional evaluation. A randomized and double-blind study found that daily consumption of 25 g of quinoa flakes, rather than corn flakes, lowered total cholesterol and LDL while increased Glutathione levels in postmenopausal women (De Carvalho et al. 2014). Qin et al. (2013) monitored the antioxidant property by DPPH assay and found that soaking of buckwheat reduced rutin concentration while elevating the quercetin and kaempferol levels, whereas steaming had the reverse effect. Buckwheat's antioxidant and inhibitory properties were reduced by roasting and drying. The effect of three pure chemicals present in bran on enzymatic activity and pancreatic lipase binding behaviour was investigated by (Li et al. 2013). The enzymatic kinetics investigation revealed that iso-quercetin, rutin and quercetin are effective lipase inhibitors in a dose-dependent form. Guo et al. (2010) investigated the impact of buckwheat protein mostly on development of breast cancer cells, as well as the mechanism. TBWSP31 treatment resulted in cell separation from culture, blebbing, plasma membrane and the production of apoptotic bodies. It caused G0/G1 arrest and stopped cell development in the S phase. This bio-function of TBWSP31 may include up-regulation of Fas activity as well as down-regulation of Bcl-2 expression. Antioxidant properties of ordinary and Tartary buckwheat was measured during germination for approximately 9 days by (Ren & Sun 2014). The strongest antioxidant as well as anti-lipid oxidation properties were found in sprouts from specimens after 9 days of germination and Tartary buckwheat performed better than normal buckwheat. Cardiovascular disease is the leading cause of morbidity and mortality worldwide, and nutrition is among the most significant risk factors. Total cholesterol level, bad cholesterol that is Low density lipo-proteins as well as triglyceride levels are risk factors for such diseases. In human patients who took 18 mL amaranth oil per day for three weeks were reported to significant decrease in total cholesterol level, TG and LDL. LDL over 130 mg/dL, HDL below 35 mg/dL and total cholesterol levels exceeding 200 mg/dL are all signs of excessive cholesterol (Martirosyan et al. 2007). Amaranth components may also benefit skin health. Phytoecdysteroids extracted from amaranth seeds have been shown to be powerful preventive agents against collagenase-related skin conditions and oxidative stress (Nsimba et al. 2008). Amaranth seeds have a mild protective effect on diabetes mellitus and fructose-induced obesity. In diabetic rats, the amaranth grain and its oil fraction drastically lowered serum glucose while increasing serum insulin levels; hence amaranth seeds are effective for treating hyperglycemia and reducing diabetes consequences. The precise components responsible for amaranth's anti-obesity and anti-diabetic properties are yet to be determined (Paśko et al. 2011).

Mode of action of amino acid that promote human health

There are many common mechanisms of action of amino acids such as, to increase the expression of intestinal β-defensin and the endogenous small cationic polypeptide that functions as a broad-spectrum antimicrobial substance. Therefore, the amino acids greatly affect the gut microbiota composition. Other modes of action of amino acids for human health promotion are discussed below.

Modulation of immune response

Many food derived compounds plays important role and are able to modulate the immune response in humans. They may mediate inflammation by changing the DNA-binding capacities of nuclear factor kappa B cells (NF-κB). NF-κB are the main effector of immune response pathways, and other transcription factors (Zhu et al 2018). Their main function is to act as central inflammatory mediator by regulating a vast array of genes involved in the immune and inflammatory responses. Liu et al (2017) reported that NF-κB responds to cytokine IL-1β, and its activation induces the expression of inflammatory cytokines, chemokines, and adhesion molecules. Thus, the control of the NF-κB pathway provides a potential strategy for preventing inflammation related diseases. Amaranth includes a high concentration of 11S as well as 7S globuli proteins, which have antihypertensive properties. The degradation of proteins by digestive enzymes produces bioactive peptides with antihypertensive action up to eight times more than unmodified proteins and a similar impact on decreasing hypertension in rats compared to drug, captopril (Priego-Poyato et al. 2021).

Free radical scavenging activity

Plant based foods are rich source of antioxidants. Li et al. (2007) reported that proteins and peptides can also plays important role and exert a protective effect. There are various mechanisms of action like free radicle scavenging, hydroperoxides, metal ion chelation and reactive oxygen species reduction for oxidation inhibition of cellular components. In addition, the typical amphipathicity of most peptides allows them to act both in aqueous and lipidic systems (Wu et al. 2003).

Regulation of cytokine and chemokine production

Recent studies showed that protease inhibitors (PIs) may play important roles in the treatment or prevention of inflammation-associated diseases, like cancer, coagulation diseases, metabolic syndrome, autoimmune diseases and obesity (de Lima et al. 2019). But the information about the protease inhibitors from pseudocereals continues to remain limited. It is known that serine proteases act as modulators of the immune system and inflammatory response by regulating cytokine and chemokine production. Abnormal functioning of serine proteases may contribute to the development of disorders derived from inflammatory cell activation that lead to immunological problems and excessive activation of inflammation and the inhibition of serine proteases by protease inhibitors plays an important role in prevention of these diseases (Clemente and Carmen 2014). There is very few literature cited on the effect of amino acid and proteins of pseudocereals which impart health benefits. Researchers need to focus on this aspect in future as pseudocereals are one of the most nutritious food item with potential health benefits.

Limitations and drawbacks of pseudocereals

Along with their beneficial aspects, pseudocereals contain many anti-nutritional compounds, such as phytates and saponins as well as molecules that may have harmful effects on the sensory attributes of derived foods. There are many challenges and limitations hampering the growth and development and proper utilization of these pseudocereals which need to be addressed are discussed below.

Antinutritional substances

Pseudocereals include various anti-nutritional substances, such as saponins, phytates etc. as well as molecules that could have a negative impact on the sensory characteristics of derived products. Quinoa seeds contain pericarp saponins that confer a bitter taste to the finished products and may hinder the absorption of zinc and iron absorption (Filho et al. 2017). Therefore, specific treatments are required to remove these non-nutritive compounds. Amaranth flour includes phytates, which inhibit the absorption of certain micronutrients such as copper, zinc, calcium, phosphorus, iron etc. (Sanz-Penella et al. 2013). To eliminate these unwanted chemicals, certain procedures are necessary (Graziano et al. 2022). Although numerous techniques for reducing phytates concentration in pseudocereals, such as malting, germination as well as soaking, which might stimulate seed phytates have been tried, but they have proven ineffectual (Rollán et al. 2019). Quinoa contains some anti-nutritional compounds such as saponins that can be removed by washing, soaking and boiling (Ponce-Garcia et al. 2021).

Bioavailability

Saponins have beneficial qualities like analgesic, anti-allergic as well as antioxidant properties. However, they also decrease vitamin bioavailability, lower the effectiveness of food conversion and can harm the cells of the small intestinal mucosa (Suarez-Estrella et al. 2018). Washing is the approach that is most frequently used in rural areas since saponins are water soluble.

Lack of awareness

Pseudocereal grains and their products are still underutilized as raw materials for the production of expanded extrudates (Mushtaq et al. 2021). Consumers are unaware about the excellent nutritional value of pseudocereals. Traditionally, they are used as staple food in many cultures but currently neglected as they are only percolated in small niches in the world food system (Pirzadah et al. 2020). To completely or partially replace common cereals in various food products, the mechanical properties of these grain alternatives should be intensively studied and evaluated.

Public awareness about the nutritional properties and health benefits of pseudocereals needs to be created through farmers fairs and exhibitions organized by Research Organizations, Krishi Vigyan Kendras, Universities and Department of Agriculture in all States of the country.

Lack of promotion and coordination among diverse stakeholders

Various type of literature such as books, newsletters, research journals and brochures on pseudocereals may be published for faster dissemination of knowledge. Preparation of recipes booklets, production guides and educational films with sound tracks in various local languages play important role for generating public awareness about the utilization and health benefits of these crops among the masses. Training programmes need to be organised from time to time by the extension workers and scientists for the farmers in order to make them aware about the latest technologies relating to these pseudocereals. Therefore, a multi-interdisciplinary approach which includes different stakeholders such as food technologists, economists, agriculturist, biotechnologist and local growers is the need of the hour to exploit these underutilized crops and to make them a real success in near future.

Marketing constraints

In case of pseudocereals more attention is required to be paid towards cultivation, processing, development of products, their value addition and ultimately marketing them to consumers. Small farmers must be encouraged to adopt contract farming model for ensuring maximum crop production, so as to make the products of these crops commercially viable in the market. Extensive studies are required to be carried out to assess the requirements of various resources for intensive and extensive crop production at varied geographical locations. These studies should also include the requirements of exact quantity of various raw materials required for efficient manufacturing process of different value added products out of these pseudocereals. All government plans formulated to increase cultivation of pseudocereals must be concurrently developed to include its marketing strategy (Mal 2022).

Lack of collaboration

The lack of collaboration between various entities like farmers, traders, processors, consumers and marketing personal need to be addressed and a strategy should be made to benefit all the stake holders. The formal and informal sectors should also be encouraged in order to develop efficient value chain for promotion of these underutilised crops. Mal (2022) reported that a strong collaboration is required to be established between the Department of Food Science/Home Science of State Agriculture Universities as well as Central Universities, other institutes of repute and food laboratories located in the country and worldwide to carryout quality analysis research work on their processing, value addition and product development.

Policies

More lucrative and goal oriented government policies should be made for enabling the use of pseudocereals in the prevalent food system. Since these grains are of high nutritive value, their use should be increased in the midday meals of the primary schools and for pregnant ladies through the Aangawadi Centres. NGOs may also be involved at various stages with the aim to boost research and development activities on pseudocereals. Local Research Centres and Cooperative Societies of farmers should be made to promote growth of pseudocereals in the areas where the geographical conditions are most suitable for their cultivation. The government should revise the incentives and subsidies given to the farmers every year to promote cultivation and expansion of the area of these underutilised crops.

Lack of improved agronomic practices

For several underused pseudocereal crops, genetic constraints exist. For example, buckwheat demonstrates self-incompatibility and is inherently cross-pollinated (Ueno et al. 2016). As a result, creating self-compatible buckwheat lines is essential for breeding as well as phenotypic enhancement. Furthermore, because pipelines for mutagenesis as well as transformation have not yet been built or are currently being improved, breeding for these grain crops is mostly dependent on natural variability. For these underused grains, there is currently little to no overlap between genomics and breeding. Overall, the absence of systematic breeding efforts aimed at increasing the use of underused grain crops in high-input agriculture systems is now a constraint (Bekkering & Tian 2019).

Conclusions

Pseudocereals should be included in the everyday diet of the global population because of their high nutritive benefits. As the nutritional qualities of these plants become better recognized, the demand for these foods is expanding. Pseudocereals have huge potential as a pure source of biologically beneficial compounds, notably peptides as well as protein hydrolysates. With advantages to human health, foundational antioxidant and antihypertensive actions, the pseudocereals based products may be highly pertinent to improve celiac patient health as well as life quality. However, further research is needed, particularly in-vivo experiments using animal models and clinical studies, to demonstrate the reported health advantages of pseudocereal protein derived peptides. The structural and chemical changes that cause changes in the components and characteristics of pseudocereals may be investigated further.

Abbreviations

SDS-PAGE	Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis
LDL	Low density lipo-proteins
GABA	Gamma-aminobutyric acid
HDL	High density lipo-proteins
TG	Triglycerides
TBWS	Tartary buckwheat water-soluble extracts
AOT	Aerosol OT sodium bis (2-ethylhexyl) sulfosuccinate
ALA	α-Linolenic acid
EPA	Eicosapentaenoic acid
NF-κB	Nuclear factor kappa B cells

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