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Proteins from Pseudocereal Grains

Asli Can Karaca

Abstract

Seeds such as quinoa, amaranth, chia, and teff are considered as potential sources of plant-based proteins for human consumption. Proteins isolated from pseudocereal grains have the potential to serve as nutritious alternatives to animal-based proteins for various food applications. Quinoa, amaranth, and chia proteins are among the most extensively studied pseudocereal proteins for the characterization of structural, physicochemical, and functional properties. This chapter will review the recent studies on composition, structural characteristics, physicochemical and functional properties of proteins isolated from pseudocereal grains, will discuss several modifications applied for improvement of functional properties and some potential end-product applications.

Keywords: pseudocereal protein, functional properties, physicochemical properties, chia, quinoa, amaranth

1. Introduction

Pseudocereal grains are considered as good sources of protein with a balanced amino acid profile. Proteins from pseudocereal grains have recently gained increasing popularity due to their nutritional, functional, and biological properties. Proteins from quinoa, amaranth, and chia are among the most extensively studied pseudocereal proteins in terms of characterization of physicochemical, functional, and biological properties. The functionality of proteins from other less known pseudocereals, such as kiwicha and cañihua, still remains to be explored. Although proteins from pseudocereal grains are indicated to show good functionality, some processes may be required to modify the structure and improve the functionality of pseudocereal proteins. Structural and functional properties of various pseudocereal proteins are recently reviewed [1–3]. This chapter presents an overview of the structural and functional properties of pseudocereal proteins, the effects of methods used for protein extraction and fractionation on protein functionality, and several methods applied for modification of structure and optimizing the functionality of pseudocereal proteins.

2. Quinoa protein

Quinoa (*Chenopodium quinoa* Willd) contains ~13–16% protein with major fractions of albumins (29–50%) and globulins (7–37%) classified based on the extraction methodology [2, 4–6]. Structural and functional properties of quinoa

protein were recently reviewed by Dakhili et al. [2]. Quinoa seed protein is reported to contain a balanced essential amino acid profile, with relatively higher amounts of lysine and methionine compared to cereals and legumes [5]. Physicochemical and functional properties of quinoa protein were investigated in recent studies to elucidate its potential for utilization as an ingredient in various food applications. It has been indicated that the method used for protein extraction has a significant effect on the composition and functionality of quinoa protein [2]. Moreover, inert physical barriers in the seed are indicated to hinder a significant portion of protein in quinoa from being extracted [6]. Van de Vondel et al. [6] recently investigated heat-induced protein denaturation and aggregation during protein extraction from quinoa using denaturing agent sodium dodecyl sulfate (SDS) and reducing agent dithiothreitol (DTT) with an aim to maximize extraction yield. The maximum protein extraction yield obtained using SDS, DTT, and/or various pretreatments was reported to be 82%, which indicated that physical barriers hinder the extraction of ~20–25% of the protein in quinoa [6].

Various physical, chemical, and biological modification methods are applied to pseudocereal proteins to improve functionality. Enzymatic hydrolysis is a commonly applied strategy to improve not only the functional but also the bioactive properties of plant-based proteins. Guo et al. [7] recently reviewed the biological activities of quinoa protein hydrolysate and peptides. In a recent study, Daliri et al. [8] applied enzymatic hydrolysis to quinoa protein concentrate with pancreatin and investigated the changes in emulsifying, foaming, and antioxidant properties. Quinoa protein concentrate was obtained from defatted quinoa flour with alkaline extraction followed by the isoelectric precipitation method. Hydrolysis with pancreatin at 40°C for 180 min was reported to result in the highest degree of hydrolysis (~19%). Fourier-transform infrared spectroscopy analysis revealed that different functional groups, such as free regions of hydroxylic amino acids, aromatic amino acids, and free amino groups, originated in the hydrolysate due to the hydrolyzing action of pancreatin. The obtained hydrolysate was reported to show better antioxidant properties in terms of 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity. Solubility, emulsifying and foaming activities of the hydrolysate were found to be higher than that of the native protein. On the other hand, the native protein showed better emulsion and foam stabilizing properties [8].

Maillard reaction is used as a tool to modify structural properties and improve the functionality and biological activity of proteins. In a recent study, Teng et al. [9] investigated the effect of glycosylation with xylose on the structural and functional properties of quinoa protein. Quinoa protein isolate (96% protein) was obtained from defatted quinoa flour with alkaline extraction followed by an isoelectric precipitation method. Glycosylation via Maillard reaction was performed by mixing quinoa protein isolate with mannose or xylose with varying proportions in phosphate buffer and heating at 60°C for 4 h. The optimum ratio of quinoa protein to monosaccharide was determined to be 2:1 based on the degree of grafting and browning index analyses. The electrophoretic profile of samples revealed that glycosylation had significant effects on the depolymerization and remodeling of molecular aggregates of quinoa protein. The specific surface area and absorption capacity of quinoa protein were indicated to increase after glycosylation. Solubility, water and fat absorption capacities, emulsifying activity, and stability of glycosylated quinoa protein were reported to be significantly higher than that of the native protein. Moreover, anti-inflammatory and anti-proliferative activities of quinoa protein were indicated to increase after the glycosylation reaction [9].

3. Amaranth protein

Amaranth (*Amaranthus* spp.) seeds contain ~13–15% protein with major fractions of albumin, globulin, and glutelin [4, 10]. Tömösközi et al. [11] investigated functional properties of amaranth protein in model systems and used casein and soy protein isolate as reference proteins for comparison. Amaranth protein isolate (80% protein) was obtained from defatted amaranth flour from two different varieties with alkaline extraction followed by the isoelectric precipitation method. Following extraction, amaranth protein isolate was separated into fractions based on the Osborne-type fractionation method. Fractions of albumin, globulin, and glutelin-type alkali-soluble residual proteins were obtained and tested for functionality. The authors observed similarities between protein profiles of soy and amaranth. However, emulsifying and foaming properties of amaranth protein and derived fractions were found to be relatively poor compared to casein and soy protein. Among the amaranth protein fractions, solubilities of albumin and globulin fractions were reported to be significantly higher than that of the residue protein. It was concluded that optimization of protein extraction and enzymatic or chemical modification of protein structure may be required for effective utilization of amaranth protein preparations as food ingredients in various end-product applications [11].

Figuerola-González et al. [12] investigated the effects of pH-shifting and ultrasonication treatments on the structure, physicochemical, and foaming properties of amaranth protein. Amaranth protein isolate (83% protein) was obtained from defatted amaranth flour with alkaline extraction-isoelectric precipitation method. Amaranth protein dispersions were prepared in distilled water (30 mg/mL, pH 7.0) and protein was modified by five different treatments—pH-shifting at pH 2.0 and 12.0, sonication (750 W) for 10 min at an amplitude of 50%, and pH-shifting (at pH 2.0 and 12.0) followed by sonication. After the modification treatments, amaranth protein dispersions were dried at 35°C for 45 h in the oven to avoid protein denaturation. Alkaline pH-shifting followed by sonication was reported to result in a significant decrease in the hydrodynamic diameter of amaranth protein. On the other hand, hydrodynamic diameter of protein was observed to increase after the acidic pH-shifting treatment. The isoelectric point of amaranth protein increased from 4.0 to 4.2 after the alkaline pH-shifting treatment and to 4.5 after the combined alkaline pH-shifting and ultrasound treatments. However, ultrasound treatment alone was reported to decrease the isoelectric point of amaranth protein to 3.5. Alkaline pH-shifting and ultrasound treatments were reported to induce changes in the secondary structure fractions of amaranth protein. Moreover, both pH-shifting treatments and combination of pH-shifting and ultrasound treatments resulted in changes in the sulfhydryl groups and disulfide bonds of amaranth protein. Both pH-shifting treatments were reported to improve the solubility of amaranth protein, where the highest protein solubility was observed in the sample treated with a combination of alkaline pH-shifting and ultrasound. The foaming capacity and stability of amaranth protein were reported to increase significantly after all treatments except for the acidic pH-shifting treatment. Moreover, treatments applied were indicated to improve the *in vitro* digestibility of amaranth protein that was attributed to the modifications in protein structure, which lead to increased accessibility to digestive enzymes [12].

Das et al. [13] investigated the effects of pH treatment and the extraction pH on the physicochemical and functional properties of amaranth protein isolate. Amaranth protein isolate was obtained from defatted amaranth flour with alkaline extraction at different pH values (9.0, 10.0, 11.0, and 12.0) followed by isoelectric precipitation

(pH 4.5). Amaranth protein isolates were subjected to pH treatments at pH 3.0–9.0 and tested for functionality. The protein content of amaranth proteins extracted at different pH values changed between 56 and 85%, where the isolate obtained at pH 9.0 showed the highest purity, solubility, and particle size but the lowest yield. The authors reported that maintaining the extraction and treatment pH values at 9.0 resulted in significant improvements in functional properties including solubility, water and oil binding capacities, emulsifying and foaming properties. Extraction and treatment at pH 9.0 were also indicated to result in better thermal properties and improved gelation characteristics. Moreover, emulsifying, foaming, and gelation properties of amaranth protein isolates were reported to be affected by the particle size, wettability, and solubility [13].

Enzymatic modification is recently applied as a useful tool to improve the functionality and biological activity of amaranth protein. Kamal et al. [14] prepared amaranth protein hydrolysates using bromelain, chymotrypsin, and pronase E enzymes at three different hydrolysis durations (2, 4, and 6 h). Bioactive peptides were identified by LC-MS-QToF analysis. Amaranth protein hydrolysates were reported to contain bioactive peptides with inhibitory properties against enzymatic markers linked with hypertension and diabetes [14].

In another recent study, Karimi et al. [15] investigated the effects of using selective hydrolyzed protein of amaranth on sourdough fermentation, bread quality, and shelf life. Amaranth protein isolate was obtained from defatted amaranth flour using the alkaline extraction-isoelectric precipitation method. Protein hydrolysates were prepared with Alcalase® treatment for 3 h. The authors reported that amaranth protein hydrolysates increased the growth of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* significantly in sourdough compared to amaranth protein isolate and amaranth flour. The use of amaranth protein hydrolysates in the sourdough formulation was indicated to result in changes in dough pH and total titratable acidity after fermentation, as well as changes in specific volume, water activity, and textural characteristics of bread. Sensory properties and shelf life of bread were reported to be improved by the addition of amaranth protein hydrolysates to sourdough [15].

Kiwicha (*Amaranthus caudatus*) is a less known pseudocereal that is indicated as a good source of protein (~13–19%) with a balanced amino acid profile [16]. The physicochemical and functional properties of kiwicha proteins still remain to be explored. There are only few recent reports in the literature on the biological activities of peptides obtained from kiwicha proteins with enzymatic hydrolysis [17–19].

4. Chia protein

The protein content of chia (*Salvia hispanica* L.) seeds changes between ~19 and 23%, and the major protein fractions are reported as globulins, albumins, glutelins, and prolamins [20]. Segura-Campos [21] characterized chia seed proteins. Protein fractions of albumins, globulins, prolamins, and glutelins were obtained based on the Osborne-type fractionation method. The main protein fraction in chia was reported to be glutelin that showed the highest water absorption and holding capacities. Significant differences were observed in emulsifying and foaming properties of fractions obtained from chia protein [21].

Julio et al. [22] prepared different protein fractions of albumins, globulins, glutelins, and prolamins from chia protein-rich fraction of chia seeds, a by-product of chia oil extraction process. The solubility profile of chia protein-rich fraction, globulins,

and prolamins was observed to be similar and made a peak at pH 9.0. On the other hand, maximum solubility was observed at pH 5.0 for glutelin and albumin fractions. Detailed emulsion characterization tests including destabilization kinetics and particle size distributions revealed that globulin fraction resulted in the most stable emulsion systems. The authors reported that higher pH values resulted in improved stability in emulsions stabilized with globulins, glutelins, and chia protein-rich fraction [22].

Urbizo-Reyes et al. [23] prepared chia protein hydrolysates with ultrasound treatment followed by microwave-assisted hydrolysis. For this purpose, chia seed mucilage and chia seed oil were extracted from the seeds prior to protein hydrolysis. Chia protein hydrolysates were prepared using Alcalase® or sequential hydrolysis with Alcalase® and Flavourzyme®. Enzymatic hydrolysis reaction was conducted using a conventional or microwave-assisted system. Chia protein hydrolysates obtained using sequential hydrolysis with microwave treatment were reported to show significantly higher *in vitro* antioxidant activity. Hydrolysates were also indicated to show antidiabetic and antihypertensive activities. Microwave treatment during hydrolysis was reported to improve the solubility profile, emulsifying and foaming properties of hydrolysates [23].

5. Teff protein

Teff (*Eragrostis tef*) contains ~13–21% protein with major fractions of glutelin, albumin, and prolamins [24]. Compared to commonly studied proteins from pseudo-cereal grains, including quinoa, amaranth, and chia, teff proteins remain to be explored in terms of functionality. Gebru et al. [24] studied the variations in amino acid profile and protein composition of white and brown teff seeds during protein extraction. Three different methods were used to obtain fractions of albumins, globulins, prolamins, and glutelins from teff seed flour. White and brown teff seeds were indicated to undergo different changes during protein extraction. Moreover, the essential amino acid content of brown teff seeds was reported to be significantly higher than that of white seeds. Extraction with tert-butanol was indicated to increase prolamins yield compared to extraction with ethanol. Glutelin was reported to be the major protein fraction in both seeds, with white seeds containing higher amounts of glutelin compared to brown seeds. The electrophoretic profile of storage proteins was observed to be different indicating the genetic variations between white and brown teff seeds [24].

Teff flour is widely used in formulations of gluten-free bread and bakery products. Adebowale et al. [25] compared the characteristics of protein fractions in three different teff types with sorghum with the main focus on bread-making quality. The major protein fraction in teff was reported to be prolamins. Aqueous alcohol-soluble protein fraction was indicated to be rich in glutamine and leucine. The authors suggested that differences in the electrophoretic profile of proteins indicated that teff prolamins are less polymerized compared to sorghum prolamins. Functional properties of teff prolamins useful in bread making were attributed to the differences in thermal profile, lower polymerization, and hydrophobicity [25].

6. Buckwheat protein

Common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) are indicated as good sources of protein (8–18% protein) with a balanced

amino acid profile [3, 26]. The major storage protein in buckwheat seeds is reported to be 13S globulin [27] and the main protein fractions were reported as albumin, globulin, and glutelin [3]. Functional and bioactive properties of buckwheat protein were recently reviewed by Jin et al. [3]. Tomotake et al. [28] compared the physicochemical and functional properties of buckwheat protein with soy protein isolate and casein. Buckwheat protein was obtained from buckwheat flour using the alkaline extraction-isoelectric precipitation method. Solubility of buckwheat protein was significantly higher than that of soy protein at pH 2.0–10.0, but lower compared to casein at pH 7.0–10.0. The stability of emulsions stabilized by buckwheat protein was observed to be lower compared to soy protein and casein-stabilized emulsions at pH 7.0–10.0. Moreover, the water holding capacity of buckwheat protein was found to be lower than that of soy protein [28].

Xue et al. [29] investigated the effects of high-intensity ultrasound treatment and Maillard reaction on structural, interfacial, and emulsifying properties of buckwheat protein. Buckwheat protein isolate was prepared from defatted buckwheat flour with alkaline extraction method followed by isoelectric precipitation. Buckwheat protein isolate-dextran conjugates were prepared via Maillard reaction combined with ultrasound treatment. The secondary and tertiary structures and surface hydrophobicity of buckwheat protein isolate-dextran conjugates obtained with ultrasonication were observed to be different than those of conjugates obtained with classical heating. As a result of the modifications in protein structure, emulsifying properties and surface activity of conjugates obtained with ultrasonication were reported to be improved compared to classical heating [29].

In another recent study, Wu et al. [30] investigated the effect of extraction pH on structure, functional properties, and digestibility of tartary buckwheat protein. Protein isolates were prepared from defatted tartary buckwheat flour using alkaline extraction at different pH values (pH 7.0–13.0) followed by isoelectric precipitation. Tartary buckwheat flour and protein isolates were separated into albumin, globulin, prolamin, and glutenin fractions based on Osborne-type protein fractionation. Protein extraction at alkaline conditions was reported to increase protein extraction yield. Increased extraction pH was indicated to decrease the albumin content of tartary buckwheat protein isolate while glutenin content increased. The solubility of isolates extracted at pH > 12.0 was observed to decrease. On the other hand, emulsion stability increased at the same conditions that were attributed to increased surface hydrophobicity. The differences observed in *in vitro* digestibility of tartary buckwheat protein isolates obtained at different pH values were related to the modifications in protein structure. The highest digestive rate was observed in isolates obtained at pH 7.0 and 8.0 [30].

In addition to functional properties, buckwheat protein and derived bioactive peptides are reported to show various biological properties, including cholesterol-lowering activity, blood pressure controlling enzyme inhibitory activity, antimicrobial and antioxidant activities that suggest the potential use of buckwheat protein and peptides as functional food ingredients [3].

7. Cañihua protein

Cañihua (*Chenopodium pallidicaule* Aellen), is a less known pseudocereal, contains ~14–19% protein and a balanced essential amino acid profile that are comparable to other commonly known pseudocereals, such as quinoa and amaranth [31].

The major protein fractions in cañihua are reported as albumin and globulin [32]. Betalleluz-Pallardel et al. [33] used response surface methodology for optimization of protein extraction conditions from defatted cañihua grain meal aiming maximized protein extraction yield. Simultaneous effects of pH (7.0–11.0), temperature (25–60°C), solvent:meal ratio (10:1–40:1), time (10–60 min), and NaCl concentration (0–2 M) on protein extraction yield were investigated. Optimum protein extraction conditions were determined as pH 10.0, 21°C, 37:1 (v/w) solvent:meal ratio, and 5 min of extraction time, which resulted in ~80% protein extraction yield [33].

Enzymatic hydrolysis was applied to cañihua protein for obtaining peptides with biological activities. Chirinos et al. [34] derived hydrolysates and peptides from cañihua protein concentrate. Protein concentrate (79% protein) was obtained from defatted cañihua meal with alkaline extraction-isoelectric precipitation method. Cañihua protein concentrate was subjected to enzymatic hydrolysis with Alcalase®, Neutrase®, and Flavourzyme® at 50°C up to 240 min. The hydrolysates obtained were purified via ultrafiltration and size exclusion chromatography to obtain three peptide fractions. The authors reported that cañihua protein can be considered as a good source of bioactive peptides with antioxidant and angiotensin-I converting enzyme (ACE) inhibitory activities. Specifically, cañihua protein hydrolysate obtained with Neutrase®-Alcalase® sequential hydrolysis for 180 min was indicated to show good *in vitro* bioactivity. Two of the purified peptide fractions composed of 3–11 amino acids were indicated to show good *in vitro* antioxidant and antihypertensive activity [34].

In another recent study, Moscoso-Mujica et al. [35] also applied enzymatic hydrolysis to cañihua protein. Cañihua flour was obtained from the seeds of two different varieties (Ramis and Cupi-Sayhua) and defatted prior to protein extraction. Protein fractions of albumins, 7S globulins, 11S globulins, and glutelins were obtained based on solubility differences and subjected to sequential hydrolysis with Alcalase® and pepsin-pancreatin. Hydrolysates with varying degrees of hydrolysis were obtained and tested for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The authors reported that among the 216 hydrolysates obtained, only 28 showed significant antimicrobial activity. It was suggested that antimicrobial peptides obtained from fractions of globulins and glutelins can potentially be utilized as novel nutraceutical ingredients [35].

8. Conclusion

Pseudocereals are indicated as good protein sources with a balanced amino acid profile. Nutritional composition and protein characteristics of pseudocereal grains change depending on the seed variety and growing conditions. Moreover, the methods used for protein extraction and fractionation affect protein structure, composition, and hence, functionality. Enzymatic hydrolysis has been shown to be a useful tool for obtaining peptides from pseudocereal proteins with biological activities, including antioxidant, antimicrobial, and antihypertensive properties. Proteins and peptides from pseudocereal grains can be potentially utilized as ingredients in innovative product formulations due to their nutritional quality, functional properties, and biological activities. More research is needed to investigate the effects of pseudocereal proteins on end-product quality to elucidate the potential and increase the utilization of pseudocereal proteins as food ingredients.

Conflict of interest

The author declares no conflicts of interest.

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