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Review

Amaranth, quinoa and chia protein isolates: Physicochemical and structural properties

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ABSTRACT

An increasing use of vegetable protein is required to support the production of protein-rich foods which can replace animal proteins in the human diet. Amaranth, chia and quinoa seeds contain proteins which have biological and functional properties that provide nutritional benefits due to their reasonably well-balanced aminoacid content. This review analyses these vegetable proteins and focuses on recent research on protein classification and isolation as well as structural characterization by means of fluorescence spectroscopy, surface hydrophobicity and differential scanning calorimetry. Isolation procedures have a profound influence on the structural properties of the proteins and, therefore, on their *in vitro* digestibility. The present article provides a comprehensive overview of the properties and characterization of these proteins.

1. Introduction

In addition to their role as a macronutrient, proteins play a key role in food structure through processes such as emulsification, foaming, gelation and dough formation. Food protein supply is presently scarce, and this situation will worsen if the world population continues to increase. As more food protein sources will be needed, research has been focusing on new alternative protein sources [1]. Thus, proteins from seeds, grains, legumes, fish, microbes, algae, and leaves are presently being evaluated [2–6].

An increasing use of vegetable protein is required to support the production of protein-rich foods which can replace animal proteins in the human diet. Otherwise, from a nutritional standpoint, plant proteins can supply sufficient amounts of essential aminoacids for human health requirements [6].

Soy is an example of how scientific research can add value and diversify the use of vegetable proteins in a wide variety of food products. While soy is the most common alternative protein source to replace animal-based protein, new food products containing proteins from other sources, such as grains, legumes and vegetables, are currently being evaluated [7].

Amaranth (*Amaranthum*), quinoa (*Chenopodium quinoa Willd*) and chia (*Salvia hispanica* L.) are non conventional sources of proteins that have been studied in recent years. They are referred to as pseudocereals, as their seeds resemble in composition and function those of true cereals. In addition, the aminoacid composition of pseudocereal proteins is well balanced, with a high content of essential aminoacids, and high bioavailability. Moreover, pseudocereals are gluten-free products, which represent a significant advance towards ensuring an adequate intake of nutrients in subjects with celiac disease [8].

Amaranth, chia and quinoa have been cultivated from tropical to subtropical regions and were important food crops to Aztec, Mayan and Incan civilizations [8,9]. However, their production and use declined significantly after the Spanish conquest. Today, these ancient crops are grown commercially in Mexico, Bolivia, Argentina, Ecuador, Guatemala and Peru [9].

The present review provides a comparative study on some aspects of amaranth, quinoa, and chia proteins based on recent research. This review compares these proteins and focuses on recent research reporting studies on: protein classification and isolation; structural characterization by means of fluorescence spectroscopy, surface hydrophobicity and differential scanning calorimetry; and *in vitro* protein digestibility.

2. Seed protein classification and characterization

Seed proteins can be classified on the bases of different criteria such as function and differential solvent solubility, among others.

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A classification mainly based on protein function divides seed proteins into three groups: "storage", "structural and metabolic" and "protective" proteins [10].

Storage proteins are those proteins which are laid down at one stage of the development for future use to supply intermediary nitrogen compounds for biosynthesis at a metabolic active stage [11,12].

Simple proteins were first classified by Osborne [13] based on the differential solubility of each fraction in both aqueous and non-aqueous solvents. This is the most widely used classification for plant proteins. The albumin fraction is obtained from a suspension in water while the globulin fraction is soluble in diluted salt solutions. Prolamins are the alcohol-soluble fraction and glutelins are the most difficult fraction to solubilize, being usually extractable with weak alkalis and acids or dilute detergent solutions [14].

The fractionation and characterization of the different groups of storage proteins from amaranth, quinoa and chia have been reported. Table 1 shows the protein fraction content of some varieties of amaranth, quinoa and chia.

The data presented in Table 1 intend to show not only the proportion of each seed protein fraction but also the evident variability found in this type of data. The solvents used in different works were not the same. In fact, salt concentrations, type of alcohol and the denaturant agents used varied, causing differences in the amount of protein solubilized in each case. Finally, various methods for protein quantification were used, and the reference method of Kjeldahl was not always the method of choice [18]. Colorimetric assays, as bicinchoninic acid, [15] or electrophoresis [16,17] were also applied to protein determination.

Sequential extraction and characterization of amaranth proteins has extensively been performed and has been revised in detail [15,20] and therefore will not be reviewed here.

Quinoa protein fractions have also been characterized in the past century [21]. Albumin fraction has been obtained from dispersion in water, while globulins have been extracted using 0.5 M NaCl. The extraction of the prolamin fraction has been the result of the suspension in a solution containing 95% of ethanol and 0.6% of β-2-mercaptoethanol. A denaturing-reducing buffer (0.0625 M Tris pH 8.1 containing SDS 2% and β-mercaptoethanol 5%) has been used to extract the glutelin fraction. These authors have found that most of the proteins in quinoa seeds can be classified into albumins or globulins. The electrophoretic profile for the albumin fraction shows both 21 definite bands and also smeared bands corresponding to molecular weight below 20 kDa. The globulin fraction contains 8 polypeptides that were visualized in SDS-PAGE: three of them correspond to molecular weights near 36 kDa, two of them to molecular weight values near 29 kDa and three to values of around 25 kDa. The electrophoresis of the prolamin fraction showed no bands and that of the glutelin fraction showed bands that correspond to other bands in albumin or globulin fractions, meaning that some insolubilization degree of these polypeptides might be caused during the protein extraction process. However, Mäkinen et al. [22] have reported that the globulin fraction, also analyzed by means of SDS-PAGE, is composed by ten polypeptides, with molecular weights ranging from less than 20 kDa up to 50 kDa. The electrophoretic pattern shown by these two groups of authors is different, probably due not only to the different electrophoretic protocol, but also to the use of different protein sources and extraction conditions.

Recently, a fractionation procedure to characterize the protein groups from chia defatted flour based on their solubility differences has been performed by Sandoval-Oliveros and Paredes-López [18]. As for the other vegetable proteins, the albumin fraction has been obtained from a suspension of chia flour in water. The pellet was resuspended in 0.5 M NaCl, in order to obtain the globulin fraction. The prolamin fraction was the result of the pellet resuspension in a 70% aqueous isopropanol solution. The resulting pellet was resuspended in a 0.1 M $Na_2B_4O_7.10H_2O$ solution (pH 10), to separate the glutelin fraction. They carried out SDS-PAGE for these samples and found that globulins are the major fraction, as shown in Table 1. The SDS-PAGE pattern shows that there are 11 bands corresponding to albumins and 19 corresponding to globulins, coinciding with five bands that correspond to polypeptides with molecular weights ranging from 50 to 200 kDa. In fact, some authors consider that it is not possible to assure the absence of some globulins in the albumin fraction; thus, they recommend considering these two groups together as one globulin + albumin fraction, for the sake of comparison [15]. The bands corresponding either to the glutelin (4 bands from 20 to 30 kDa) or prolamin (2 bands around 30 kDa) fractions visualized in the SDS-PAGE correspond to bands observed in the globulin fraction. According to Fukushima [23], the glutelin fraction may be classified either as a globulin or glutelin fraction, since he attributes the difficulty in the solubilization of these proteins to the denaturation caused by the processing of the samples (effect of solvents, temperature, among others).

On the other hand, Olivos-Lugo et al. [19] have reported a significantly different proportion of fractions in Mexican chia seeds, with prolamins and glutelins being the most abundant fractions. They found not only different proportions of each fraction, but also a 12.3% of completely insoluble protein. In our opinion, these differences in solubility could be attributed to the different methods applied to obtain the defatted flour, as pointed out above [23].

Table 2 shows the composition of aminoacids of amaranth, quinoa and chia seeds and their protein fraction

Amaranth albumin and globulin are relatively rich in essential Lys aminoacid and sulfur aminoacids, while glutelins are a source of Phe + Tyr and Leu [15]. Barba de la Rosa et al. have reported a higher content of Val in the albumin and globulin fraction [16].

The essential aminoacid content in quinoa is higher than that in amaranth. The high content of Lys in quinoa seed is higher than that recommended by FAO/WHO (5.5 g/100 g protein), while the Met content is lower than FAO/WHO recommendations [17].

Chia seeds exhibit a high content of sulfur, aspartic, and glutamic aminoacids. The high level of aspartic and glutamic acids is of interest to the food industry due to the role they play in hormonal regulation and immunological stimulation, respectively. In chia seeds, FAO/WHO coverage is 100% for sulfur aminoacids, while, for the other essential aminoacids, it ranges from 52 to 76% and from 66 to 126% in children and adults, respectively. For the globulin fraction, this coverage ranges from 27 to 210% in infants and 34 to 288% in adults [18].

Table 1
Proportion of the Protein Fractions (g fraction/100 g raw protein).

	Amaranth (%)		Quinoa (%)		Chia (%)	
Variety/type/Origin	Azteca	Waxy	Tango	Faro	Chionacalyx	Jalisco
albumins	51	47.2	13.4	13.2	17.3	3.9[a]
globulins	15.9	18.9	51.4	60.2	52	7[a]
prolamins	2	0.7	ND[b]	ND[b]	12.7	53.8[a]
glutelins	31.1	23.8	5.9	3.2	14.5	23.0[a]
reference	[15]	[16]	[17]		[18]	[19]

[a] By this procedure of extraction 8.8% of insoluble proteins were obtained.

[b] ND: not determined.

Table 2
Aminoacid composition (g/100 g crude protein).[a]

	Amaranth [15]					Quinoa [17]				Chia [18]	
	seed	alb	glob	glut	prol	seed	alb	glob	glut	seed	glob
Ala	4.3	4.1	3.5	4.1	5.6	9.3	6.8	7.3	5.4	2.68	3.94
Arg	9.3	11.4	11.9	10.9	8.7	14.0	8.2	18.6	9.6	4.23	9.42
Asx	7.7	9.1	9.1	7.0	7.8	9.2	7.8	3.1	9.5	4.73	7.29
Glx	16.0	21.5	18.8	11.9	10.8	9.9	12.5	11.0	11.8	7.08	24.3
Gly	5.9	5.8	7.7	5.7	5.9	3.8	9.0	6.8	5.9	2.28	7.36
His*	3.4	2.6	2.6	2.7	2.0	3.2	2.4	5.2	3.3	1.37	4
Ile*	3.8	3.4	3.8	4.8	4.3	3.5	5.2	4.7	5.7	2.42	3.01
Leu*	6.0	5.3	5.8	7.7	9.6	6.6	7.5	6.4	8.9	4.15	4.44
Lys*	6.1	7.1	7.5	4.5	7.2	6.9	7.5	3.2	5.4	2.99	1.54
Met* + Cys*	5.6	5.4	3.8	3.9	3.3	4.2	1.6	2.8	2.4	2.78	5.75
Phe* + Tyr*	6.6	7.5	6.1	9.2	8.7	8.2	10.1	13.5	10.5	3.88	10.93
Pro	4.8	4.2	3.4	5.0	3.8	9.4	5.0	4.5	4.4	1.99	10.64
Ser	5.6	4.9	5.9	6.9	6.2	4.9	4.6	4.1	5.1	2.62	6.93
Thr*	4.5	3.0	3.5	4.7	6.3	3.9	5.2	4.2	4.7	1.8	6.23
Val*	5.1	3.4	4.3	4.3	3.9	4.6	6.1	4.5	6.0	2.85	3.59

[a] alb, glob, glut and prol referred to as albumin, globulin, glutelin and prolamin fraction, respectively.

3. Protein isolation

A method to obtain a sample containing high levels of vegetable protein is the treatment of the defatted flour with alcohol or diluted acid to solubilize carbohydrates, obtaining a product called "concentrate". Whereas this methodology is commonly applied to obtain soy protein concentrates [24], its application to the obtention of amaranth, quinoa or chia protein concentrates has not yet been reported. Alkaline solubilization followed by isoelectric precipitation is the traditional and most commonly used method to extract proteins from seeds. A protein extraction procedure from defatted seed flour consists mainly of the solubilization of proteins in diluted alkali followed by its isoelectric precipitation in diluted acid [25]. This methodology has also been combined with treatments including different types of membrane separations.

Proteins may be induced to important structural changes as a result of alkali and acid treatments. In fact, the isolation procedure influences the functional properties of extracted proteins [26,27]. Therefore, the study of the isolation conditions is of great importance to diminish undesirable effects. The isolation conditions for amaranth and quinoa proteins by isoelectric precipitation have been studied.

The optimization of the isoelectric precipitation in order to obtain amaranth protein isolates (API) has been reported by Salcedo-Chávez et al. [28]. The extraction was performed in the pH range from 7.8 to 9.2, while the acid precipitation was carried out in the range from 4.3 to 5.7. Protein content, colorimetric evaluation and thermal characterization by DSC were performed to analyze the protein isolates obtained by each isolation procedure. Solubilization proved to be optimal at pH 8 or 9.2, whereas the best precipitation pH was 5.7.

Other studies reported that amaranth proteins were solubilized at pH 9 or 11 from defatted flour, and were then precipitated at pH 5 [29]. Isolates obtained at pH 11 were less soluble than those obtained at pH 9.

Research studies recently published have reported the use of pH 9 and 5 for the extraction and precipitation steps, respectively, during isoelectric precipitation of amaranth proteins [30–34].

A quinoa protein isolate (QPI) was obtained by Aluko and Monu [35] from quinoa seed flour dispersed in a 0.015 M NaOH solution, followed by its precipitation at pH 4.5. Abugoch et al. studied the extraction of quinoa proteins at pH 9 and 11, precipitating at pH 5 [36]. By DSC they determined that proteins denatured at pH 11, while some degree of structure remained when solubilized at pH 9. Protein solubility was lower when isolates were obtained by high alkali solubilization. However, the isolates obtained by solubilizing at pH 9 or at pH 11 had similar water holding capacities. A further study applied solubilization at pH 9 and precipitation at pH 5 to obtain protein isolates from different quinoa varieties [37].

As regards chia protein extraction, solubilization was carried out at pH 12 and precipitation at pH 3 [19]. The precipitate was heated at 90 °C and held at this temperature for 10 min. A further purification of this chia protein isolate (CPI) has also been carried out by Timilsena et al. [38]. After the precipitation step at pH 3, the precipitate was recovered through centrifugation, re-suspended in deionized water and neutralized. Then, the protein isolate was suspended in water at pH 12, followed by precipitation with cold acetone. The purified isolate was then recovered by centrifugation.

The optimization of the dry fractionation procedure to obtain protein-rich fractions from chia defatted flour has been reported by Vázquez-Ovando et al. [39]. As it has been described, it consists in sifting the flour using a mesh (140 μm screen) in which the high protein content fraction passes through and the fiber-enriched fraction is retained. The aforementioned authors reported that a great proportion of globulins were present, and that the protein-rich fraction contained 446.2 g/kg of crude protein. They also highlighted the simplicity of this method, as well as the lack of effluent production. This methodology has recently been used by other authors to obtain chia protein-rich fractions in order to study their physicochemical, functional and biological properties [40–42].

Another method, known as micellization, is based on the ability of proteins to form agglomerates with a micellar structure. This takes place by decreasing the ionic strength of the solution in which they are solubilized [43].

A comparative study among these methodologies has been applied to API [44]. On the one hand, according to the micellization method reported, proteins were extracted from defatted meal with 0.8 M NaCl at pH 7, for 2 h at 35 °C. After centrifugation, the supernatant was concentrated by ultrafiltration and then was then diluted 1:12 with distilled water. The concentrate was stirred for 2 h at 25 °C. Proteins were recovered by centrifugation and then freeze-dried. On the other hand, the isoelectric precipitation was carried out from a suspension of amaranth defatted meal in water, at pH 9. After stirring at 25 °C for 2 h, the suspension was centrifuged at 4 °C, and the supernatant was precipitated at pH 4.5. The precipitate was resuspended in water, adjusted to pH 7, and freeze-dried. The authors reported that protein isolation by micellization methodology resulted in a lower

content and lower yields of proteins, which were less soluble than those obtained by isoelectric precipitation. However, less protein denaturation has been highlighted as an advantage of the micellization procedure.

Nothing has yet been reported about quinoa and chia isolation by micellization methodology.

4. Structural characterization

Food protein structure provides the molecular bases to various aspects of protein functionality and ability to interact with other food ingredients. Functional properties depend not only on the protein primary sequence, but also on their conformation [45].

4.1. Fluorescence spectroscopy

There are numerous reports on the use of fluorescence spectroscopy to study protein structure. Three aminoacid residues -Phe, Tyr and Trp- contribute to the UV-fluorescence of proteins. However, Trp dominates protein emission as it absorbs at the longest wavelength and displays the largest extinction coefficient. Moreover, the energy absorbed by Phe and Tyr is usually transferred to the Trp residues of the same protein. In most studies, protein fluorescence is often excited near 280 nm or at longer wavelengths and, as a result, Phe is not excited [46].

Trp residues emission spectra are sensitive markers of the protein environment. In an apolar environment, a blue-shifted emission is observed, whereas when Trp residues are exposed to polar environments [46], the emission shifts to longer wavelengths.

The structure of the soluble fraction of amaranth and quinoa proteins was studied by fluorescent spectroscopy. To our knowledge, there are no reports of fluorescent spectroscopy studies of chia proteins so far.

An API (obtained by solubilization at pH 9 and precipitation at pH 5) was studied at pH 7.5, exciting at 290 nm, and it showed its maximum emission wavelength at 345 nm [47]. Acid denaturation of this amaranth isolate resulted in a decrease in the fluorescence emission and a red shift in the maximum wavelength (to 353 nm).

The effect of pH on amaranth proteins solubilized at pH 9 and 11 has also been analyzed by fluorescence spectroscopy [36]. Fluorescence intensity increased with increasing pH in both samples. The authors reported that this might be due to conformational changes or to solubilization of new protein species. Only in the case of proteins solubilized at pH 9, a significant red-shift of the higher emission wavelength was reported at acid pH, which is attributed to a higher Trp exposition to the solvent.

QPI solubilized at pH 9 (Q9) and at pH 11 (Q11) were studied at pH 7, exciting at 290 nm, which resulted in maximum emission wavelengths of 334 and 348 nm, respectively [36]. The authors reported that Trp residues of these isolates are solvent-exposed, showing higher exposition in the isolate obtained at higher alkaline pH. Acidification of the quinoa isolates at pH 3 resulted in a decrease in fluorescent intensity, the decrease for Q11 being higher than that for Q9. Moreover, the wavelength of maximum emission red-shifted to 348 and 360.5 nm for Q9 and Q11 isolates, respectively. The authors explained that this could be a consequence of Trp being exposed to a more polar environment either by exposition to an acidic environment or by the protonation of the acid amino-acid residues. This would also suggest the denaturation of proteins in both isolates [36].

Gorinstein et al. [48] have compared the fluorescence properties of amaranth and quinoa globulins at pH 7.5, exciting at 295 nm, thus, the typical spectra of Trp were obtained. The spectrum of amaranth globulins showed a maximum emission peak near 341.5, whereas a more apolar environment for the Trp residues was found for quinoa globulins, which resulted in a wavelength of maximum emission of 333.5 nm. Based on their results, these authors have suggested that amaranth globulins contain higher amounts of Phe and Tyr than quinoa globulins; even though shoulders indicating the presence of Tyr were found for quinoa globulins at an excitation wavelength of 275 nm. However, this is not in concordance with the aminoacid composition of the globulin fractions of amaranth and quinoa reported by Segura-Nieto et al. [15] and Thanapornpoonpong et al. [17].

Urea-induced denaturation of amaranth and quinoa globulins produced a decrease in the fluorescent emission. The degree of denaturation of these proteins in urea 8 M, calculated based on the fluorescence intensity, was 25% for amaranth globulins and 18% for quinoa globulins [48]. Such differences may be due to the differences in the amounts of aminoacids and the sulfur bridges existing among these proteins [49]. A red-shift in the maximum of emission wavelength and a decrease in energy transference from excited Tyr to Trp was found for amaranth globulins in the presence of urea [48].

4.2. Surface hydrophobicity

Aggregation and denaturation are frequently occurring processes in proteins. Surface hydrophobicity (S_0) is a useful tool to study these phenomena and is often measured using the fluorescent probe ammonium 8-(anilino)-naftalene-sulfonate (ANS) according to the method proposed by Kato and Nakai [50], with slight modifications.

S_0 determinations of amaranth and quinoa proteins have been reported and in all cases, they were calculated as the initial slope of fluorescence intensity *vs*. protein concentration plots. There have been no reports on S_0 of chia proteins so far.

The S_0 of API decreased when the concentration of protein increased from 1 to 5% or 10%, which was attributed to the formation of protein aggregates at high concentration through protein hydrophobic patches [51]. This article also reported the pressure-induced denaturation of amaranth proteins and the characterization of these denatured proteins through the determination of S_0.

The S_0 of acid treated amaranth proteins has also been studied and it has proved to be lower than in native proteins, since hydrolyzed peptides do not expose hydrophobic patches large enough to bind ANS [47].

Mäkinen et al. [52] have studied the S_0 of native and thermally treated quinoa isolates. The authors have reported an increase in S_0 as a result of protein unfolding when the protein isolate was heated at pH 8.5. When it was heated at pH 10.5, S_0 was similar to that in the native isolate.

Thermally-induced aggregation of quinoa globulins was studied [22]. This type of aggregation can occur either by disulfide crosslinking of thiol groups or by increased hydrophobic exposure. In order to quantify their relative importance, the effect of pH and heating time on the S_0 of quinoa globulins was analyzed. S_0 increased after heating at 100 °C for the first 5 min at all pH conditions studied (6.5; 8.5 and 10.5). When heated further, a slight increase in S_0 was observed at pH 6.5 and 8.5, indicating unfolding of the tertiary structure. However, at pH 10.5, globulins showed a decrease in their S_0, confirming that the thermally induced aggregation of quinoa proteins at this pH is driven by hydrophobic interactions. This assumption may be consistent with the results obtained by Mäkinen et al. [52].

4.3. Differential scanning calorimetry (DSC)

The thermal characterization of amaranth, quinoa and chia proteins has been carried out by DSC and has already been reported. The denaturation peak temperature (T_d), denaturation temperature range (ΔT_d) and denaturation enthalpy (ΔH_d) have been determined. Many authors have reported the ΔH_t, *i.e.*, the total enthalpy for thermograms with different endothermic peaks, rather than one ΔH_d for each thermal transition.

Table 3 shows the thermal characterization for amaranth, quinoa and chia proteins.

Among the vegetable proteins revised in this work, amaranth has been the most extensively studied. In the past century, several studies on amaranth proteins have been reported and their thermal behavior was characterized based on DSC [27,53,54]. However, in recent years a vast number of API conditions have been studied and their effect on the thermal stability of the protein fractions was analyzed by DSC.

Two endothermic peaks have been reported for API, $T_{d,1}$ of about 75 °C, and $T_{d,2}$ near 100 °C [47,51,55,56]. No significant differences among the denaturation peak temperatures were observed among the studies revised in this work. The albumin fraction showed one peak at 64 °C; however, this value, which is the principal fraction, is not representative for the protein isolate behavior. On the other hand, the two peaks observed are in agreement with those obtained for the glutelin fraction [27].

When Abugoch et al. [36] analyzed a QPI by DSC, the thermogram obtained showed two peaks, but the authors reported only a T_d at 98.8 °C. This peak was also observed at 97 °C when alkaline extraction was carried out at different pH values (8–10), and it was assigned to the denaturation of the globulin fraction [57]. However, a previous article reports a different T_d (58 °C) for quinoa globulins [54].

Olivos-Lugo et al. [19] and Sandoval-Oliveros et al. [18] have reported the thermal characterization of chia proteins and significant differences may be observed in the T_d and ΔH_d obtained for each extracted fraction. The main difference among these results could be due to the procedure of protein hydration prior to analysis. Sandoval-Oliveros et al. have studied the same protein/water ratio for all protein fractions (5 mg protein/15 μL water), while Olivos-Lugo et al. have reported that water to protein mass ratios were 0.3, 0.4, 0.9 and 0.9 for albumins, globulins, prolamins and glutelins, respectively. It is clear that the water content of these protein fractions was not the same. As a consequence, differences in their thermal behavior may be observed. In spite of these disparities, in both cases the authors have revealed that chia protein fractions showed high thermal stability. Timilsena et al. [38] have reported a single broad peak with high T_d (97 °C) for the CPI. The thermal stability of chia proteins was improved when it interacted with chia seed gum (108.6 °C) by complex coacervation. An even higher T_d (119.3 °C) was reported in the same study when the authors analyzed the complex coacervate cross-linked by transglutaminase.

As regards flours, different results have been obtained. Abugoch et al. [58] reported quinoa flour thermograms with two endothermal peaks. The authors pointed out that the lowest T_d (65.7 °C) obtained corresponded to starch gelatinization and the other (98.9 °C) resulted from protein denaturation. This latter transition showed a similar T_d to that reported in another study [36] for the QPI. Amaranth native and defatted flours have also been studied by DSC [59]. Starch gelatinization and protein denaturation were analyzed by the thermo-

Table 3
Thermal characterization of amaranth, quinoa and chia protein isolates and fractions.

Sample	Conditions	Number of peaks	T (°C)		ΔH (J/g)		ΔTd(°C)	Reference
API	AE + IP different cultivars	2	among 68.1–69.2		among 95.2–96.6		among 0.43–0.71	[33]
	AE + IP pH 8, ionic strength 0.06 M	2	74.6 ± 0.4	101.7 ± 0.7	8.3 ± 0.2		NR	[55]
	AE + IP pH 8, ionic strength 0.5 M	2	70.2 ± 0.2	99 ± 1	10.51 ± 0.04		NR	
	AE + IP pH 2, ionic strength 0.06 and 0.5 M	Endotherms not detected						
	AE + IP	2	69.87	101.57	NR		NR	[60]
	AE + IP	2	70.7 ± 0.1	98.6 ± 0.1	4.09 ± 0.35	5.69 ± 0.57	NR	[51]
	AE + IP	2	75.5 ± 0.2	101.8 ± 0.3	7.6 ± 0.6		NR	[47]
	AE + IP + acid treatment	Endotherms not detected[VB3]						
Amaranth Globulin	Protein fractions extracted sequentially by a methodology based on solubility differences	2	Near 65 (NR)	94	Near 20		NR	[27]
Amaranth Albumin-1		1	64		near 2.5		NR	
Amaranth Albumin-2		1	94		near 10		NR	
Amaranth Glutelins		2	70	96	below 10		NR	
QPI	AE (pH 9) + IP	2	Near 90 (NR)	98.1 ± 0.1	12.4 ± 1.6		85.6–103.1	[36]
	AE (pH 11) + IP	Endotherms not detected						
	AE (pH 8, 9, 10 during 16 hs) + IP	1	97		Up to 10.2		NR	[61]
	AE (pH 11 during 16 hs) + IP	Endotherms not detected						
Quinua globulins	Dispersion at 0.5 M NaCl	1	58.0 ± 1.5		10.0 ± 1.6		NR	[54]
CPI	Alkaline extraction followed by isoelectric precipitation	1	97.2		3.2		75.3–114.1	[38]
Chia albumins	Protein fractions extracted sequentially by a methodology based on solubility differences	1	80.1 ± 0.66		2.70 ± 0.17		60.7–101	[19]
			103.6 ± 0.7		12.6 ± 0.8		96.0–118.8	[18]
Chia globulins		1	125 ± 1.55		3.60 ± 0.27		116–136	[19]
	104.7 ± 0.2		4.7 ± 0.9		94.3–116.6	[18]		
Chia prolamins		1	70.4 ± 0.46		1.91 ± 0.02		61.3–79.8	[19]
	85.6 ± 0.6		2.3 ± 0.2		72.1–93.2	[18]		
Chia glutelins		1	105 ± 50		2.74 ± 0.51		94.5–115	[19]
	91.3 ± 0.8		6.8 ± 0.1		76.0–104.9	[18]		

grams obtained and exhibited a similar behavior to that reported for quinoa flour. So far, there have been no reports on chia flour studies by DSC.

5. *In vitro* protein digestibility

The aminoacid profile of a protein is of great importance when evaluating its nutritional quality. However, protein digestibility, defined as the amount of protein absorbed into the body relative to the amount that was consumed, is the major factor in determining the actual availability of its aminoacids [62]. As bioassays are expensive and time-consuming procedures, different *in vitro* digestibility methodologies have been developed in the last century [63–66].

The *in vitro* protein digestibility method used by Vazquez-Ovando et al. [39] for a chia protein-rich fraction obtained by dry processing was a modification of a previously reported method [62]. Briefly, a multienzyme solution was added to a pH-adjusted protein suspension and incubated with stirring in a water bath at controlled temperature. A rapid decrease in pH was produced and was recorded at 10 min to estimate *in vitro* digestibility. The authors reported that a low *in vitro* digestibility was obtained (77.53%), probably because of protein denaturation during the fat extraction process. A similar methodology was carried out later in another research [18]. The *in vitro* digestibility of the globulin fraction was 82.5 ± 1.1%, slightly higher than the one obtained for the defatted flour (78.9 ± 0.7%). Chia- defatted flour showed similar results in both research studies. Pepsin digestion in 0.1 M HCl at 37 °C was carried out for a CPI and for the defatted flours. The reaction was stopped by adding trifluoroacetic acid and the total nitrogen content of the soluble fraction was examined by using the Kjeldahl method. *In vitro* digestibility proved to be 49.4 ± 1.58% and 28.4 ± 0.2% for the CPI and the defatted flour, respectively [19]. The authors explain that the thermal treatment and alkalization applied during protein extraction may account for the enhanced digestibility reported for the isolate, when compared with the flour. It is to be noted that the digestibility of this defatted flour was significantly lower than that previously mentioned. Not only have different digestion procedures been applied, but also different quantification methodologies have been used; thus, comparison among them should be avoided.

The protein digestibility of amaranth wholemeal flours from different genotypes was determined by using the digestibility method proposed by Hsu et al. [62] and ranged from 73.0 to 76.2% (the average protein digestibility reported was 74.2%) [67,68]. A slight increase was reported for amaranth protein concentrates (from 78.7 to 82.0%) [68].

The *in vitro* digestibility has also been studied for QPI from different genotypes [69]. The authors reported that these protein isolates exhibited excellent *in vitro* digestibility (between 75.95 ± 0.29 and 78.11 ± 0.43%). Even though Vázquez-Ovando et al. [39] reported that low protein digestibility was obtained for the CPI, the results obtained were significantly similar to those reported for quinoa proteins. Similar methodologies have been applied in both research studies. When considering the human digestibility of some types of proteins, such as egg, milk, meat and fish, it can be seen that their digestibility is not high. However, these values were comparable to the digestibility of other vegetable proteins such as rice, corn, and beans [70].

Excluding the study reported by Olivos-Lugo et al. [19], it may be concluded that *in vitro* digestibility proved to be similar for chia and amaranth defatted flours. Moreover, similar results have been reported for QPI and API, which is also comparable to that reported for the globulin fraction of chia proteins.

6. Conclusion

This article provides an overview of recently published research on composition and structural properties of amaranth, quinoa and chia proteins as well as their *in vitro* digestibility.

Quinoa grains contain higher amounts of essential aminoacids than amaranth and chia grains; however, a significant variability in the aminoacid profile as well as in the fractionation products of the proteins has been found among the revised articles. The most widely studied method for protein isolation is alkaline extraction followed by isoelectric precipitation. Moreover, several conditions (pH, ionic strength and salts) have been assayed in the isolation of amaranth and quinoa proteins. Chia proteins have also been obtained by dry fractionation and API have also been isolated by micellization.

Spectroscopy and thermal characterization, carried out by DSC, indicate that isolation procedures have a profound influence on the structural properties of the proteins. As a general rule, chia proteins are more stable than amaranth and quinoa proteins, which show similar stability.

It is to be noted that the *in vitro* digestibility of these proteins is high, making them suitable for human consumption.

Further systematic research on quinoa and chia proteins should be directed to elucidate the relationship between isolation conditions and structural characteristics. A deeper understanding of this relationship would allow choosing the most appropriate isolation conditions in order to obtain the functional properties desired.

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