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# Study of Some Physicochemical and Functional Properties of Quinoa (Chenopodium Quinoa Willd) Protein Isolates

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The amino acid composition and the physicochemical and functional properties of quinoa protein isolates were evaluated. Protein isolates were prepared from quinoa seed by alkaline solubilization (at pH 9, called Q9, and at pH 11, called Q11) followed by isoelectric precipitation and spray drying. Q9 and Q11 had high levels of essential amino acids, with high levels of lysine. Both isolates showed similar patterns in native/SDS-PAGE and SEM. The pH effect on fluorescence measurements showed decreasing fluorescence intensity and a shift in the maximum of emission of both isolates. Q9 showed an endotherm with a denaturation temperature of 98.1 °C and a denaturation enthalpy of 12.7 J/g, while Q11 showed no endotherm. The protein solubility of Q11 was lower than that of Q9 at pH above 5.0 but similar at the pH range 3.0−4.0. The water holding capacity (WHC) was similar in both isolates and was not affected by pH. The water imbibing capacity (WIC) was double for Q11 (3.5 mL of water/g isolate). Analysis of DSC, fluorescence, and solubility data suggests that there is apparently denaturation due to pH. Some differences were found that could be attributed to the extreme pH treatments in protein isolates and the nature of quinoa proteins. Q9 and Q11 can be used as a valuable source of nutrition for infants and children. Q9 may be used as an ingredient in nutritive beverages, and Q11 may be used as an ingredient in sauces, sausages, and soups.

**KEYWORDS: Quinoa protein isolate; amino acid composition; physiochemical property; functional property**

## INTRODUCTION

Quinoa is a lost crop of the Incas, a cereal-like crop with high seed yield. The seed protein content is high (about 15%), and its essential amino acid balance is excellent because of a wider amino acid spectrum than cereals and legumes (*4*), with higher lysine (5.1−6.4%) and methionine (0.4−1.0%) contents (*1–3*). Quinoa proteins may be one of the more promising food ingredients, capable of complementing cereal or legume proteins, and there is the potential for the production of protein concentrates from dehulled quinoa seeds, which could be used as raw materials in the food industry. The main protein fractions in quinoa grain are albumins and globulins (*5, 6*). Brinegar et al. (*5, 6*). studied the molecular structures of quinoa globulin and albumin, and they reported that both proteins are stabilized by disulphide-type bridges. The use of protein isolates has increased in the food industry because of different factors such as high protein level, good functionality, and low content of antinutritional factors (*7*). In spite of the high nutritional value of quinoa proteins because of the high quality of amino acids, their use as food ingredients in the form of protein isolates depends largely on their functional properties, which are related to their structural characteristics and, together with their composition at various alkaline pH values, depend on the extraction pH and the composition and degree of unfolding of the protein isolates, and they may be controlled by different extraction conditions (*8–12*). The processes applied to obtain protein isolates have in common the use of alkaline media (pH 8−11) for the solubilization of proteins and acidic media (pH 4−6) for their isoelectric precipitation (*13*). The proportions of the different fractions in a protein isolate and their particular functional and nutritional properties depend on the method of preparation used; for example, for amaranth protein isolates obtained by two distinct methods, alkaline extraction-isoelectric precipitation and micellization, the alkaline extraction had a greater protein yield and protein content than micellization (*7*). The composition, yield, and degree of unfolding of protein isolates could be controlled by choosing different combinations of extraction and precipitation pH (*8, 14, 15*). The functional properties of food proteins are important in food processing and food product formulation, and some of these properties are related to hydration, such as water holding capacity (WHC), water imbibing capacity (WIC), and solubility. The purpose of

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**Table 1.** Amino Acids Composition of Quinoa Protein Isolates Q9 and Q11, Soy Protein Isolate (SPI)[a], and Casein[b]

| | mg/g of protein | | | |
|---|---|---|---|---|
| amino acid | Q9 | Q11 | SPI | casein |
| arginine | 99.7 ± 2.7[d] | 89.4 ± 1.7[e] | 41.0 | 37.0 |
| aspartic acid | 80.1 ± 0.9[d] | 70.6 ± 0.9[e] | 118.1 | 63.0 |
| cystine | 5.5 ± 1,4[d] | 6.8 ± 0.5[d] | 0.6 | 0.4 |
| glycine | 53.8 ± 1.1[d] | 50.5 ± 0.7[e] | 38.6 | 16.0 |
| glutamic acid | 163.6 ± 5.4[d] | 145.4 ± 8.4[e] | 212.9 | 190.0 |
| histidine | 25.8 ± 0.3[d] | 23.0 ± 0.6[d] | 29.0 | 27.0 |
| isoleucine | 43.3 ± 0.8[d] | 39.1 ± 0.7[e] | 44.8 | 49 |
| leucine | 73.6 ± 1.4[d] | 69.2 ± 1.3[e] | 70.0 | 84.0 |
| lysine | 52.5 ± 0.6[d] | 52.1 ± 1.0[d] | 53.9 | 71.0 |
| methionine | 21.8 ± 0.2[d] | 23.5 ± 0.7[e] | 9.3 | 26.0 |
| phenylalanine | 44.9 ± 0.7[d] | 41.4 ± 0.7[e] | 53.0 | 45.0 |
| serine | 52.1 ± 0.6[d] | 47.6 ± 0,2[e] | 54.8 | 46.0 |
| threonine | 43.9 ± 0.9[d] | 42.7 ± 0.4[d] | 41.0 | 37.0 |
| tryphtophan[c] | 38.5 | 35.6 | | 14.0 |
| tyrosine | 35.4 ± 0.5[d] | 35.6 ± 0.3[d] | 37.1 | 55.0 |
| valine | 50.6 ± 0.9[d] | 47.5 ± 0.9[e] | 44.1 | 60.0 |
| alanine | 38.2 ± 0.7[d] | 37.4 ± 0.6[d] | 38.3 | 27.0 |

[a] Data from Tang et al. (*12*). [b] Data from Wang et al. (*42*). [c] Data from Comai et al. (*43*). [d] Different letters means significant differences between raw ($p < 0.05$). [e] Different letters means significant differences between raw ($p < 0.05$).

the present work was to obtain quinoa protein isolates and study the physicochemical and functional properties. The amino acid composition was also determined.

## MATERIALS AND METHODS

**Plant Materials.** The seeds of quinoa (Chenopodium quinoa Willd) (commercial cultivar) used in this work were grown in the VI Region of Chile and supplied by Mr. Pablo Jara.

**Flour Preparation.** The seeds were washed many times with cold water to remove saponins until there was no more foam in the wash water, and they were then dried at 50 °C until 15 ± 1% moisture. The quinoa seeds were ground and sifted through a 60 mesh (*16*). The flour so obtained was defatted for 24 h with hexane in a 10% (w/v) suspension with continuous stirring, air-dried at room temperature, and finally stored at 4 °C until used. Protein, moisture, nonfiber carbohydrates, and ash content were determined according to official methods (*17*); the factor used for proteins was 5.85 (*18*).

**Amino Acid Analysis.** Amino acids were determined by precolumn derivatization with diethyl ethoxymethylenemalonate and reversed-phase high-performance liquid chromatography (HPLC) with spectrophotometric detection at 280 nm according to Alaiz et al. (*19*). The HPLC system consisted of a Merck-Hitachi L-6200A pump (Merck, Darmstadt, Germany) with a Rheodyne 7725i injector with a 20 $\mu$L sample loop, a Merck-Hitachi L-4250 UV−vis detector, and a Merck-Hitachi D-2500 chromato-integrator. Separation of the derivatives was attained using a Nova-Pack $C_{18}$ (300 × 3.9 mm id, 4 $\mu$m particle size; Waters, Milford, MA). Sample preparation and chromatographic conditions were according to Ortiz et al. (*20*).

**Protein Isolates.** The isolates were extracted by solubilizing at pH 9 and pH 11 and precipitation at pH 5. For that purpose, at each pH level, the flour was suspended in water (10% w/v), and the pH was adjusted to the required value (pH 9 or 11) with 2 N NaOH. The suspensions were stirred for 30 min at room temperature and then centrifuged at 9000*g* for 20 min. The supernatants were adjusted to pH 5 with 2 N HCl and then centrifuged for 20 min at 9000*g* and 4 °C. The precipitates were resuspended in water, neutralized with 0.1 N NaOH, and spray-dried. The isolates so obtained were termed Q9 and Q11. Each preparation was softly homogenized, and the resultant suspensions (at 15% w/w) were fed to a B-191, Büchi spray dryer (Switzerland) equipped with a centrifugal wheel atomizer. The spray-dryer was operated at an inlet temperature of 117 ± 5 °C, an outlet temperature of 70 ± 5 °C, and an atomization pressure of 1.4 kg/cm$^2$. The powders obtained were kept at 4 °C until analyzed. The soluble protein content of the isolates and fractions was measured according to the Bradford method (*21*).

**Electrophoresis.** All electrophoretic runs were performed in gel minislabs Mini Protean III Model, Bio-Rad Laboratories, polyacrylamide gel electrophoresis (PAGE). Sodium dodecyl sulfate (SDS)-PAGE and nondenaturing PAGE (5%) were performed according to Laemmli (*22*). SDS-PAGE gels contained 12% (w/v) acrylamide (5% acrylamide stacking gels), and native PAGE contained 6% (w/v) acrylamide (4% acrylamide stacking gels). The molecular mass standard was from Bio-Rad Precision Plus Protein (61−0373 N): 10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kDa. Nondenaturing gels contained 6% (w/v) acrylamide (4% acrylamide stacking gels). The sample loading buffer contained 0.124 M Tris-HCl (pH 6.8), 15% (v/v) glycerol, and (for SDS-PAGE only) 2% (w/v) SDS. For reducing conditions, 5% (v/v) $\beta$-mercaptoethanol (2-ME) was added, and the samples were heated (100 °C, 3 min). Protein bands were stained with Coomassie Brilliant Blue R.

**Differential Scanning Calorimetry (DSC).** Runs were performed in a Mettler Toledo 822 calorimeter using Mettler Toledo Star System software. For the runs, 20% w/w suspensions of isolates were prepared in distilled water. DSC samples consisted of hermetically sealed aluminum pans filled with 12−14 mg suspensions. They were run at a rate of 10 °C/min from 27 to 120 °C, and a double, empty pan was used as a reference. After each run, the dry matter content was determined by puncturing the pans and heating them overnight at 107 °C. The denaturation parameters were calculated with the software equipment, with the denaturation temperature ($T_d$) being considered as the value corresponding to the maximum transition peak, whereas the transition enthalpy ($\Delta H$) and cooperativity, represented by the width at half-peak height ($\Delta T_{1/2}$) values were calculated from the area below the transition peaks. Determinations were performed in duplicate.

**Fluorescence Spectroscopy.** Protein isolates were dispersed in pH 3 through pH 11 buffers at a constant ionic strength of 0.5 (*23*), the dispersions were gently stirred for 1 h at room temperature and centrifuged at 8500*g* for 30 min at 15 °C, and the soluble fraction of protein was normalized at 0.2 mg/mL by the Warburg method (*24*). Fluorescence spectra for the samples were recorded on a Perkin-Elmer luminescence spectrometer LS 50 B at room temperature. The excitation wavelength was 290 nm, and the emission spectra were recorded as the average of three spectra from 310 to 500 nm by using a scan speed of 30 nm/min.

**Scanning Electronic Microscopy (SEM).** The SEM of samples was obtained by using the critical point drying method. In this method, the samples were desiccated with acetone and then saturated with $CO_2$. After that, they were sputter-coated (Balzer, CD004) with gold and examined in a Jeol Scanning Microscope (Jeol, JSC 6400, Akishima, Tokio, Japan) at 20 kV.

**Solubility.** The solubility of the isolates was studied by preparing 1% w/v suspensions over a pH range from 3 to 11 (*23*). The isolates were dispersed in different buffers to 0.5 ionic strength; the dispersions were gently stirred for 1 h at room temperature and centrifuged at 8500*g* for 30 min at 15 °C. Soluble proteins in the supernatant were determined by Bradford's method (*21*), and solubility was expressed as a percentage of the total protein. Determinations were performed in triplicate.

**Water Holding Capacity (WHC).** Isolates were dispersed in buffer solutions over the pH range 3−11 (*23*) at 1% w/v using a vortex mixer and then stirred for 30 min and at 25 °C. After the mixture was thoroughly wetted, the samples were centrifuged (8500*g* for 30 min). After the centrifugation, the amount of added distilled water resulting in the supernatant liquid in the test tubes was recorded, and the soluble proteins in the supernatant were determined by Bradford's method (*21*). WHC (g water/g sample) was calculated as

$$\mathrm{WHC} = (m_2 - (m_1 - m_3))/m_1$$

where $m_1$ is the weight of the dry sample (g), $m_2$ is the weight of the sediment (g), and $m_3$ is the weight of the soluble protein from the supernatant (g). Triplicate determinations were made for each sample.

**Water Imbibing Capacity (WIC).** WIC was determined according to Añón et al. (*26*). It was expressed as milliliters of water imbibed per gram of sample. The total to imbibed water ratio was calculated according to the method of Añón et al. (*26*). Determinations were performed in triplicate.

**Table 2.** Comparison of Essential Amino Acids Content of Q9 and Q11 to SPI[a], Casein[b], and FAO/WHO Suggested Requirements[c]

| | mg/g of protein | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | amino acids content | | | | FAO/WHO suggested requirement | | | |
| amino acid | Q9 | Q11 | SPI | casein | 1 yr old | 2−5 yr old | 10−12 yr old | adult |
| histidine | 25.9 | 23.1 | 29.0 | 27 | 26 | 19 | 19 | 16 |
| isoleucine | 43.1 | 39.3 | 44.8 | 49 | 46 | 28 | 28 | 13 |
| leucine | 73.2 | 69.6 | 70.0 | 84 | 93 | 66 | 44 | 19 |
| lysine | 52.3 | 52.4 | 53.9 | 71 | 66 | 58 | 44 | 16 |
| SAA[d] | 27.8 | 30.3 | 9.9 | 26 | 42 | 25 | 22 | 17 |
| ARM[e] | 80.0 | 77.3 | 90.1 | 100 | 72 | 63 | 22 | 19 |
| threonine | 43.6 | 42.8 | 41.0 | 37 | 43 | 34 | 28 | 9 |
| tryphtophan | 35.6 | 35.6 | | 14 | 17 | 11 | 9 | 5 |
| valine | 50.4 | 47.8 | 44.1 | 60 | 55 | 35 | 25 | 13 |

[a] Data from Tang et al. (*12*). [b] Data from Wang et al. (*42*). [c] Data from Friedman and Brandon (*30*). [d] Sulfur-containing amino acids, methionine and cystine. [e] Aromatic amino acids, phenylalanine and tyrosine.

**Statistical Analysis.** Data were analyzed by analysis of variance and significance of differences between means by the Tukey's multiple-range test (Statgraphic version 4.0). A $p$ level of 0.05 was used to determine significance.

## RESULTS AND DISCUSSION

**Chemical and Amino Acid Composition.** The dry weight composition of Q9 was the following (% w/w): protein, 77.2 ± 0.15; nonfiber carbohydrate, 18.8 ± 0.5; ash, 3.0 ± 0.2; water, 6.1. The composition of Q11 was the following (% w/w): protein, 83.5 ± 0.2; nonfiber carbohydrate, 11.9 ± 0.2; ash, 3.5; water, 6.8. The protein content of Q11 was higher than that of Q9 ($p < 0.05$), probably because of the high initial pH extraction, and those values were similar to those of soy and amaranth protein isolates (*8, 11, 26, 27*). On the other hand, the protein content of Q9 and Q11 was higher than that of sesame isolate (*29*).

The amino acid composition is presented in **Table 1**, and for comparison, soy protein (SPI) and casein were included because both are considered good sources of amino acids (*12*).

Q9 had higher levels of arginine, cystine, leucine, methionine, threonine, and valine than those of SPI and similar contents of other amino acids. Compared to casein, Q9 had similar or higher levels of histidine, phenylalanine, arginine, aspartic acid, cystine, glycine, serine, threonine, and alanine, while the content of the other amino acids was lower.

Q11 presented similar or higher contents of arginine, glycine, leucine, serine, threonine, alanine, and the sulfur-containing amino acids, methionine and cystine, than those found in SPI and casein.

The composition of essential amino acids of Q9 and Q11 is shown in **Table 2**. SPI, casein, and the FAO/WHO suggested requirements (*30*) are also included for comparison. Q9 had essential amino acid levels similar to those of SPI and similar or higher levels of histidine, sulfur-containing amino acids, threonine, and tryptophan than those of casein. Q11 had a higher content of sulfur-containing amino acids, threonine, and tryptophan than SPI and casein. According to the FAO/WHO suggested requirements for one-year-old infants, Q9 had high levels of tryptophan (38.5 mg/g of protein) and aromatic amino acids (phenylalanine and tyrosine, 80 mg/g of protein) and similar histidine, isoleucine, threonine, and valine contents. In comparison, only lysine in Q9 and Q11 is a limiting amino acid for two- to five-year-old infants or children, while all the essential amino acids of this protein are sufficient according to FAO/WHO suggested requirements for 10- to 12-year-old children. Both quinoa isolates studied showed a good amino acid profile and can be used as good sources of protein for feeding infants and children.

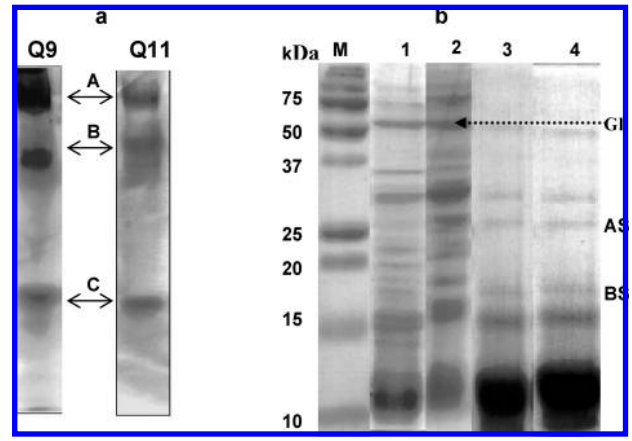


**Figure 1.** (**a**) Native-PAGE: Isolate Q9 and Q11. A, B, and C are the protein profile. (**b**) SDS-PAGE profiles of quinoa isolate proteins: in no reducing conditions (lane 1, Q9 and lane 2, Q11); with 2-ME (lane 3, Q9 and lane 4, Q11). Gl inside the figure indicates the globulin; AS and BS within the figure indicate the acidic and basic subunits, respectively. M is the protein standard.

**Electrophoresis Pattern.** Quinoa isolate proteins were compared by native-PAGE, and three major similar protein profiles (A, B, and C, indicated in **Figure 1a**) were found by native-PAGE for both isolates. The SDS-PAGE profiles of Q9 and Q11 are shown in **Figure 1b**. In the presence or absence of the reducing agent $\beta$-mercaptoethanol (2-ME), Q9 and Q11 showed similar protein constituents (**Figure 1b**, lanes 1−4). Like other 11S proteins, isolated quinoa proteins also include so-called "chenopodin", which includes acidic (AS) and basic (BS) subunits (*6, 23, 31–33*). Quinoa globulin (Gl **Figure 1b**, lanes 1 and 2) was composed of AS and BS with molecular weights (MWs) of about 30 and 20 kDa, respectively (**Figure 1b**, lanes 3 and 4). The peptides with MWs of less than 20 kDa correspond to the albumin components, according to Brinegar et al. (*5*).

**Fluorescence Spectra.** A further examination of the structural changes in Q9 and Q11 was made by monitoring changes in the fluorescence emission spectra of tryptophan residues in soluble fractions (SF) of Q9 and Q11, as shown in **Figures 2** and **3**. Between pH 5 and 11, the fluorescence spectrum of SF-Q9 showed a broad peak with a maximum at about 335 nm (**Figure 2**); this was different from SF-Q11, which showed a broad peak with its maximum at 347 nm (**Figure 3**) under the same conditions. It is known that the emission maximum of tryptophan buried within the protein is well below 330 nm, whereas that of solvent-exposed tryptophan is observed at approximately 335 nm and above (*34*). The emission maxima of 335 nm observed for SF-Q9 and 347 nm for SF-Q11 therefore

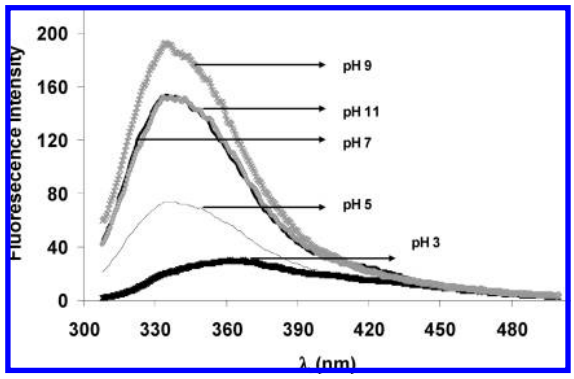


**Figure 2.** Effects of pH on the fluorescence emission spectrum of quinoa SF of isolate protein, Q9.

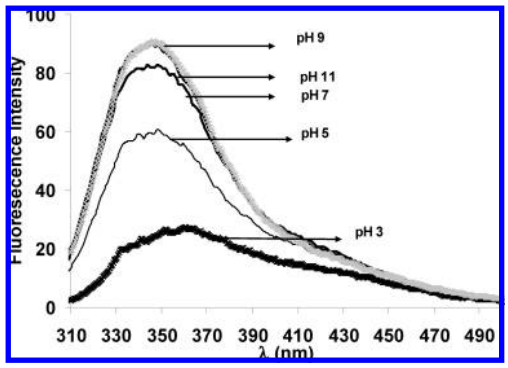


**Figure 3.** Effects of pH on the fluorescence emission spectrum of quinoa SF of isolate protein, Q11.

suggest exposure to the solvent of tryptophan residues in the protein between pH 5 and 11. Reducing the pH to 3 caused a notable decrease in fluorescence intensity (**Figures 2** and **3**) and a red shift to a higher wavelength. The quenching of the fluorescence intensity and a red shift in the maximum occurred as the pH was decreased for the SFs, and the effect was pronounced at pH 3. The quenching of the fluorescence intensity is due to the exposure of tryptophan residues to the polar environment from the interior hydrophobic environment (*35*). The maximum shifted from 334 nm at pH 7 to 366.8 nm at pH 3 for SF-Q9 and from 348 to 360.5 nm for SF-Q11. The red shift in both cases is indicative of a more polar environment of the tryptophan residue due to exposure of tryptophan to the acidic environment or addition of protons to the residues in the immediate environment of tryptophan (*35, 36*). The observed red shift in the emission maximum at acid pH would suggest denaturation of the SF for both isolates (*36*). Fluorescence analysis showed that, when both isolates are subjected to extreme pH values, they undergo a landslide of the maximum toward the red and a significant decrease of the fluorescence intensity, with this decrease greater for Q11.

**Differential Scanning Calorimetry (DSC).** The thermal transition of Q9 and Q11 was analyzed by DSC; their thermograms are shown in **Figure 4**. Q11 showed no endotherm, indicating that this protein isolate was denatured due to its extreme pH extraction (*8*) The thermogram of Q9 shows an endotherm between 85.6−103.1 °C; this endotherm showed two peaks, and this may be attributed to the chenopodin component in different states. It was found that it has a principal denaturation temperature ($T_d$) at 98.1 ± 0.1 °C and denaturation enthalpy of 12.4 ± 1.6 J/g. The $T_d$ obtained indicates that Q9 had a thermal stability, just like other globulins (*23, 31, 37*). On the other hand, the Q9 endotherm showed a low cooperativity by the large $\Delta T_{1/2}$ of 11.6 ± 0.7 °C (*31, 38*). When analyzing

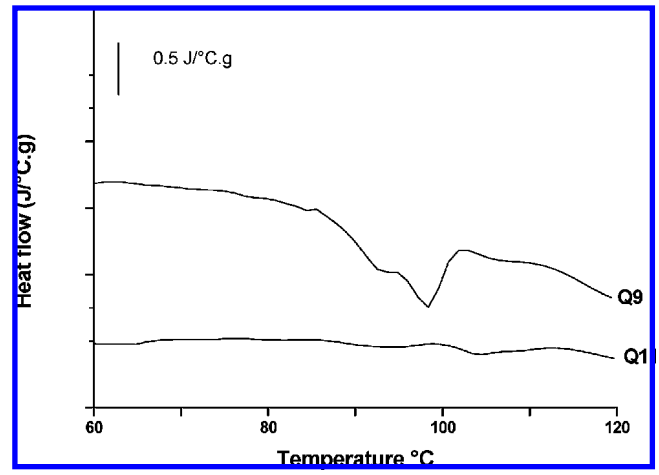


**Figure 4.** DSC thermograms of Q9 and Q11.

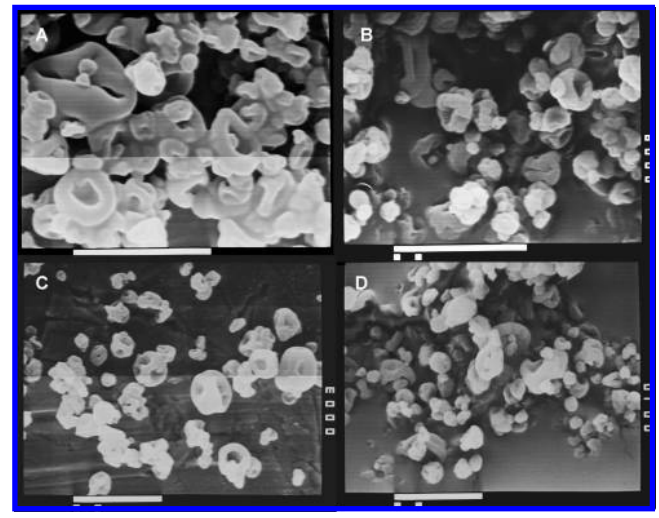


**Figure 5.** Scanning electronic microscopy of Q9 (**A**), Q11 (**B**) magnified 4.500× and Q9 (**C**), Q11(**D**) magnified 3.000×.

both isolates by DSC, Q11 was denatured, in contrast to Q9 which presented some degree of structure, indicating that an extreme alkaline pH has a negative effect on the structure.

**Microstructure.** Scanning electron microscopy is a useful tool in food science. In our present results, it has been useful to reveal preliminary structural characteristics of Q9 and Q11. Both isolates have similar microstructural characteristics: both show smooth surface and donut shape (**Figure 5**). In the literature, Añón et al. (*26*) described that small spherical and porous particles were observed for soy protein isolates. On the other hand, the size distribution of the Q9 and Q11 microparticles observed was wide (**Figure 5**). The pH extraction for Q9 and Q11 did not cause evident alterations in the microstructure appearance; this could be attributed to Q9 and Q11 having the same kind of protein fractions.

**Protein Solubility (PS).** The PS of Q9 and Q11 was minimum in the 3.0−4.0 pH range and increased above pH 5.0 (**Figure 6**). For Q9, in the 5.0−6.0 pH range, about 77% of protein was solubilized, and at above pH 7.0, the PS increased to more than 85%. On the other hand, with Q11, only about 22% of protein was solubilized in the 5.0−7.0 pH range; above pH 7.0, PS increased only to 41%, and the data suggest that Q11 was composed of aggregated or denaturalized protein molecules, as we can observe by the DSC results, and the differences found were between the fluorescence intensity spectra for Q9 and Q11. In comparison, Q9 had a different PS

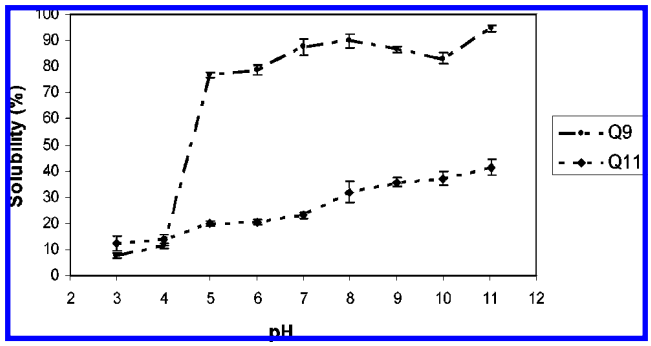


**Figure 6.** The pH profiles of protein solubility for Q9 and Q11.

**Table 3.** Water Imbibing Capacity

| isolate | WIC (mL of water/g of isolate) |
|---|---|
| Q9 | $1.7 \pm 0.1^a$ |
| Q11 | $2.6 \pm 0.03^b$ |
| soy protein isolate native[c] | 1.70 − 4.20 |

[a] Different letters means significant differences between raw ($p < 0.05$). [b] Different letters means significant differences between raw ($p < 0.05$). [c] Data from Jovanovich et al. (*40*).

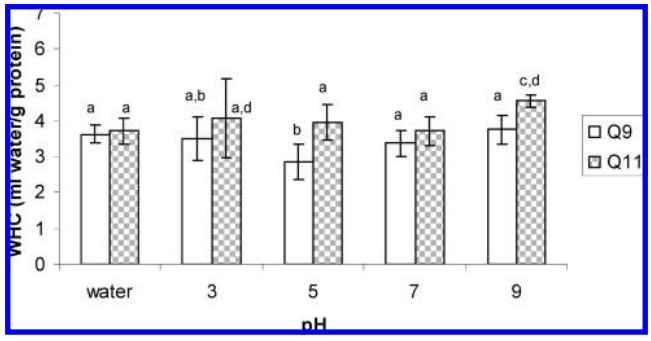


**Figure 7.** WHC of Q9 and Q11 at different pH values. The letters above the graphic within the figure presents the significant ($p < 0.05$) difference as compared with pH and between isolates.

profile than Q11, and the PS of Q9 was higher than that of Q11 above pH 5 (**Figure 5**). These differences found could be attributed to the extreme pH treatments in protein isolates and the nature of quinoa proteins. Q9 had higher solubility relative to Q11 because of a greater degree of aggregation of Q11. Q9 showed good solubility in both acidic (pH 5−6) and alkaline pH regions, which is an important characteristic for food formulations.

**Water Imbibing Capacity.** The WIC of quinoa protein isolates is shown in **Table 3**. As it can be seen, Q11 had a higher WIC than Q9. The WIC is determined mainly by the content and the level of hydration of the insoluble fraction of a protein isolate. On the other hand, the isolates with better solubility exhibit lower WIC because they contain a low proportion of insoluble protein fraction (*39*). In this context, the low WIC of Q9 can be explained by the higher solubility at acidic and basic pH, and consequently, the lower insoluble fraction. The WIC values of quinoa isolates are in a range reported for soy protein isolates (*39, 40*).

**Water Holding Capacity.** The WHC of Q9 was similar to Q11 values (**Figure 7**); there was no significance difference ($p > 0.05$) between both isolates in almost all pH ranges (pH 3−7), except to pH 9. Q11 had a superior WHC at pH 9 (4.5 mL of water/g of sample). There was no relationship between the solubility and WHC of Q9 or Q11. The high solubility of Q9 (>87%) does not correlate with its high WHC (between 2.8 and 3.7), suggesting that a significant reduction of solubility is not necessary to produce a high WHC (*41*). The quinoa protein isolates had a similar WHC (2.8−4.5 mL of water/g of sample) to that of soy protein isolates (4.3 mL of water/g of sample) (*12*). The WHC was not affected by the pH, indicating that the polar groups causing those interactions with the water molecules were available on the surface. Q11 had a WIC value greater than Q9, a property related to the degree of aggregation.

Q9 and Q11 had similar essential amino acid composition, and most of the essential amino acids are sufficient for the FAO/WHO suggested requirements of infants or children. However, Q9 shows good protein solubility, and Q11 shows much poorer protein solubility. In contrast, Q11 shows the highest WIC, and both isolates showed similar values of WHC. It can be said that pH does have an influence on the structure−function relation found for the quinoa isolates, with extraction at pH 9 favoring their structure and solubility, while Q11 was more denatured than Q9 and had lower solubility, with a favorable WIC.

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