Physicochemical and Functional Characterization of Protein Isolated from Different Quinoa Varieties (*Chenopodium quinoa* Willd.)

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ABSTRACT

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The objectives of this work were to investigate the nutritional and physicochemical characteristics as well as the functional properties of quinoa protein isolates (QPI) from different varieties, and to determine their potential use of such protein isolates in food products. Proteins were isolated by isoelectric precipitation at pH 5 from quinoa flour, and the QPI had a protein percentage of over 85%. The comparison of the flours and QPI electrophoretic profiles indicated that the extraction method allowed isolating practically all proteins of each variety. All the varieties analyzed had high lysine content, compared with cereals, and the essential amino acid content of Bolivian varieties was higher than varieties from Peru. The pH value affected the solubility and foaming capacity, and the magnitude of effects depended on the variety. Cluster analysis showed a strong influence of variety source and amino acid composition on protein physicochemical and functional properties; samples from Bolivia (cluster 2) were characterized as having the highest thermal stability, oil binding capacity, and water binding capacity at acid pH; samples from Peru (cluster 1) had the highest water binding capacity at basic pH and foaming capacity at pH 5. QPI presented a potential as an alternative vegetable protein for food application, in particular for vegetarian and vegan diets.

Quinoa has been used by people of the Andean region for hundreds of years. Andean crops grow mainly in areas with unfavorable climatic and ecological conditions for other crops and adapt to a wide range of environmental conditions (either cold mountain climates or subtropical conditions, at sea level or in high mountains). Quinoa (*Chenopodium quinoa* Willd.) has great resistance to frost, soil salinity, drought, diseases, and pests and can tolerate a wide range of pH (from 4.8 to 9.5) in the soil (Jacobsen et al. 2003). This seed has a high nutritional profile with respect to cereals, and the Food and Agriculture Organization of the United Nations (FAO) identified it in 2013 as an alternative for increasing the production of quality food to feed the world's population in a context of climate change and for those countries that suffer from food insecurity. Quinoa is considered a source of protein, fiber, polyunsaturated fat, and a wide range of minerals and vitamins (Hager et al. 2012). Also, quinoa proteins are rich in lysine, threonine, and methionine (essential amino acids), compared with cereals (Vega-Gálvez et al. 2010; Stikic et al. 2012). However, the nutritional composition of quinoa varies among ecotypes owing to strong genetic variability and the influence of environmental conditions (Repo-Carrasco et al. 2003).

The nutritional importance of proteins lies in their amino acids, which are necessary for the growth and development of human beings, as well as in their structural and functional properties and in the numerous protein–protein and protein–other component interactions that occur during food processing (Lluch et al. 2001).

At present, there is a tendency in the food industry to use vegetable protein sources to obtain desirable characteristics for consumers and facilitate industrial processing (texture, appearance, and flavor) in the development of traditional and novel food items. In addition, vegetable protein can produce bioactive peptides during digestion with beneficial health properties (Galvez et al. 2001).

Also the food industry often uses certain acids, alkalis, and salts to improve nutritional values, to control acidity and alkalinity, to trap gas, and to retain aromatic and flavoring agents (Arogundade 2006); therefore, proteins should have acceptable functional properties under the various processing conditions.

Different sources of plant protein have been investigated to determine their use in the food industry (Day 2013). However, the functional characteristics of proteins from different quinoa genotypes have not been extensively studied.

The study of alternative sources of protein, such as the production of isolated protein from quinoa, allows generating knowledge and information for proposing new functional foods.

The aim of the present work was to study the nutritional, physicochemical, and functional properties of quinoa protein isolates (QPI) obtained from six quinoa varieties cultivated in Bolivia and Peru to determine their potential use in food products.

MATERIALS AND METHODS

Materials. Three sweet quinoa varieties (Chucapaca, Kurmi, and Jacha Grano [Jacha]) from Bolivia and three (two sweet: Rosada de Huancayo [Rosada] and Pasankalla; and one bitter: Blanca de Hualhuas [Blanca]) from the Huancayo region of Peru were analyzed (cereals program of Universidad Nacional Agraria La Molina). Bolivian quinoas are commercial varieties from the PROINPA Foundation. Kurmi was obtained from Amarilla de Maranganí variety as maternal parent and accession L-57(85) as paternal parent, pedigree 61(93)/1/1/1-4/1-8/8/1-4/M/M/. Jacha Grano was obtained from accession 1489 and Huaranga variety crossing, pedigree 26(85)/4/1/2/1/1/1/M/1-6/1-10/M/ (www.proinpa.org). Chucapaca variety was obtained after crossing 0086 and 005 quinoa accessions. Rosada de Huancayo variety, which results from the crossing of Rosada de Junín (El Mantaro) and Real Púrpura (from Bolivia), was spread with UNC-U20P-69 key. Quinoa Blanca de Hualhuas was obtained by selection of its segregates; it originated in UNC-H-20-P-69 and was registered with UNC-H 20 B-74 key. Pasankalla is a variety obtained in 2006 by selection of ecotypes from Caritamaya (Ácora district) in the province of Puno. The process of improvement was carried out from 2000 to 2005 in the field of the Agricultural Experimental Station Illpa-Puno by a group of researchers at the National Program of Agricultural Innovation in Andean Crops.

Quinoa grains were subjected to a manual cleaning process to remove impurities and chaff, and then quinoa seeds were washed with cold tap water to remove saponin. Subsequently, the samples were dried at 40°C until the moisture content reached 15 ± 2 g/100 g. Quinoa flour was made in a blade mill (Oster, Boca Raton, FL, U.S.A.) and was then sieved (60 mesh).

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Protein Isolated from Quinoa. Samples of flour were defatted with hexane (1:3 w/v) for 24 h at 22 ± 2°C. Defatted quinoa flour was suspended to 15% w/v with distilled water; pH was adjusted to 9 with 2M NaOH. The suspension was stirring for 30 min at room temperature and then was centrifuged for 20 min at 10,000 × *g* (20°C). The precipitate was discarded, and the pH of the supernatant was adjusted to 5 with 2M HCl; the mixture was again centrifuged at 5°C for 20 min at 10,000 × *g* (Abugoch et al. 2008). The obtained precipitate (QPI) was resuspended with distilled water at 20% w/v and neutralized with 0.2N NaOH. The suspension was dialyzed with distilled water for 24 h to 5°C. Then the QPI was frozen at –80°C and lyophilized.

Characterization of Protein Isolates. Protein content was determined by Kjeldahl analysis according to AACC International Approved Method 46-13.01 (N × 6.25), moisture content by AACCI Approved Method 44-19.01, and ash content by AACCI Approved Method 08-01.01. The carbohydrate content was calculated by difference. QPI color was determined with a spectrophotometer (508d, Minolta, Osaka, Japan) in CIELAB space, for which L^* indicates lightness, a^* indicates hue on a green (–) to red (+) axis, and b^* indicates hue on a blue (–) to yellow (+) axis.

Amino Acid Composition. Amino acids were analyzed by the HCl hydrolysis HPLC method, following official method 994.12 of AOAC International (1999) for amino acid analysis in feed. HPLC (Perkin Elmer, Waltham, MA, U.S.A.) with UV-visible spectrophotometric detection was used to identify and quantify amino acids. QPI (0.25 g) was hydrolyzed for 24 h at 100°C with 25 mL of 6M HCl. The samples (100 µL) were collected and dried under a stream of N_2 at room temperature. The precipitate was resuspended in 1 mL of 1M borate buffer (pH = 9) containing 0.02% sodium azide and was agitated for 1 min in a vortex mixer. Then 0.8 µL of derivatizing reagent (diethyl ethoxymethylenemalonate) was added, samples were heated at 50°C for 50 min, and each was stirred for 5 min. The samples (20 µL) were injected into the chromatographic system (Zorbax Eclipse Plus C18 column, 4.6 × 150 mm, 5 µm particle size; Agilent Technologies, Santa Clara, CA, U.S.A.). The separation of amino acid derivatives was attained by using as the mobile phase a binary gradient of 25mM sodium acetate buffer (pH = 6) and acetonitrile. The chromatographic conditions were flow rate of 0.7 mL/min and room temperature. The derivatives were detected at 280 nm. The amount of each amino acid present in the sample was calculated with reference to the standard calibration curve; the result was expressed in g/100 g of protein. Tryptophan not was determined because this amino acid was destroyed by acid hydrolysis. Determinations were performed in triplicate.

SDS-PAGE Analysis. Total protein extractions of flour (30 mg) and isolated protein (10 mg) were carried out with 1.3 mL of buffer solution (0.063M Tris-HCl, pH 6.8, 1.5% w/v SDS, 3% 2-mercaptoethanol, 10% v/v glycerol, and 0.01% w/v bromophenol blue). Suspensions were stirred for 2 h at room temperature. Then all samples were heated to 100°C for 5 min and centrifuged at 10,000 × *g* for 10 min (25°C). The resulting supernatant was the total extraction of proteins from flour and protein isolates under reducing conditions.

Electrophoresis was performed on 70 × 80 × 0.75 mm gels with a Mini Protein II cell (Bio-Rad Laboratories, Richmond, CA, U.S.A.) according to the method of Laemmli (1970). A stacking gel and a separator, with 4 and 12% acrylamide, respectively, were used. Molecular weight (MW) standards were obtained from Bio-Rad (SDS-PAGE MW standards, broad range). The bands were stained with 0.25% Coomassie brilliant blue in a methanol/water/acetic acid (4:5:1 v/v) solution and then destained.

Functional Properties of Protein Isolates. *Protein Solubility.* QPI solubility was studied by preparing 1% w/v suspensions of protein over different pHs. QPIs (10 mg) were suspended with 1 mL of the following 0.1M buffer solutions: $H_3C_6H_5O_7/Na_3C_6H_5O_7$ of pH 3 and 5; Na_2HPO_4/NaH_2PO_4 of pH 7; and $Na_2CO_3/NaHCO_3$ of pH 9 and 11. The ionic strength was adjusted at 0.4M with NaCl. All samples were stirred for 30 min at room temperature, and suspensions were centrifuged at 8,500 × *g* for 10 min at 25°C (Abugoch et al. 2008). The protein content of the supernatant was determined by the Lowry method (Waterborg 2009). The protein solubility was expressed as a percentage of the total protein. Determinations were made in triplicate on each sample and at each pH.

Foaming Capacity and Stability. Foaming capacity and stability were determined according to the method of Sathe and Salunkhe (1981). First, 50 mL of 1% (w/v) protein isolate solution of appropriate pH (buffer previously described; pH 3, 5, 7, 9, and 11) was whipped for 3 min in an Osterizer blender, and then the foaming capacity and foaming stability were determined:

$$\mathrm{FC}(\%) = \frac{V \text{ after whip} - V \text{ before whip}}{V \text{ before whip}} \times 100 \qquad (1)$$

$$\mathrm{FS}(\%) = \frac{V \text{ after 60 min stand} - V \text{ before whip}}{V \text{ after whip} - V \text{ before whip}} \times 100 \qquad (2)$$

where FC is foaming capacity, FS is foaming stability, and V is volume. Each determination was performed in triplicate.

Water Binding Capacity (WBC). QPIs were suspended in buffer solutions (pH 3, 5, 7, 9, and 11, previously described) at 1% w/v. They were stirred for 30 min and centrifuged at 8,500 × *g* at room temperature for 10 min. Soluble proteins of the supernatant were determined by the Lowry method (Waterborg 2009). WBC was expressed as g of water/g of protein isolate and was calculated with the following equation:

$$\mathrm{WBC} = \frac{m_2 - (m_1 - m_3)}{m_1} = \frac{\text{g of water}}{\text{g of PI}} \qquad (3)$$

where m_1 is dry sample weight, m_2 is weight of the sediment, m_3 is weight of soluble protein in the volume extracted, and PI is protein isolate. Determinations were performed in triplicate.

Oil Binding Capacity (OBC). Protein isolate (100 mg) was mixed with sunflower oil (1 mL) for 1 h at 30°C. The mixture was centrifuged (1,500 × *g*, 30 min), and oil was decanted. The oil retained was weighed. OBC was expressed as g of oil/g of protein isolate and was calculated with the following equation:

$$\mathrm{OBC} = \frac{m_2 - m_1}{m_1} = \frac{\text{g of oil}}{\text{g of PI}} \qquad (4)$$

where m_1 is dry sample weight, m_2 is sediment weight, and PI is protein isolate. Determinations were performed in triplicate.

Thermal Stability. Thermal properties were determined by differential scanning calorimetry (DSC822E, Mettler Toledo, Greifensee, Switzerland). QPIs were suspended in distilled water (20% w/v), and 40 mg of the suspension was weighed in a 100 µL hermetically sealed aluminum capsule. Samples were heated from 25 to 120°C at 10°C/min. Onset temperature (T_o, °C), denaturation temperature (T_p, °C), and denaturation enthalpy (ΔH, J/g) were obtained with STARe version 9.0x software (Mettler Toledo). Each run was made in triplicate.

Statistical Analysis. All results were compared by analysis of variance and significant differences between means by Fisher's LSD test (significance level of $P < 0.05$). Cluster analysis was performed on the basis of Euclidean distances, by using average linkage sorting with the maximum cluster number arbitrarily set to two. Clusters were made by using variables for the amino acid content and the content according to classification (basic, acid, polar, and hydrophobic) (INFOSTAT Statistical Software, Facultad de Ciencias Agropecuarias, UNC, Argentina).

To analyze whether amino acid composition and variety origin influenced the physicochemical characteristics and functional properties of protein isolates, an analysis of variance of thermal properties, color, foaming capacity, solubility, and oil and water retention between the two clusters obtained was conducted.

RESULTS AND DISCUSSION

Characterization of Protein Isolates. The composition of protein isolates obtained is presented in Table I. In all varieties, protein content was higher than 85%, whereas ash content was less than 2% and carbohydrate content less than 14%. Pasankalla isolate presented the highest purity grade (95% protein). Isolate composition (more than 85% protein and no more than 2% ash) was comparable to those obtained by other authors who used a similar extraction method (Brinegar and Goundan 1993; Abugoch et al. 2008) and protein isolates from amaranth (Martínez and Añón 1996) and cashew nut (Yuliana et al. 2014).

The analysis of the amino acid composition showed that quinoa grain has a good balance with a high content of lysine amino acid (between 3.2 and 4.7 g/100 g of protein) and methionine (between 6.0 and 9.0 g/100 g). Significant differences ($P < 0.05$) between varieties for all essential amino acids were found (Table II).

Amino acid scores provide a useful estimate of the protein quality of foods and are acceptable substitutes for biological assays. The chemical score or amino acid score is the proportion of each essential amino acid contained in a protein compared with a suitable reference protein or amino acid pattern (milligrams of amino acid per gram of test protein/milligrams of amino acid per gram of protein in the reference pattern). The amino acid that shows the lowest proportion is called the limiting amino acid, and the ratio obtained is the score (Pellett and Young 1980). Of all the amino acids determined, the limiting amino acids, compared with the values recommended by the FAO for schoolchildren, were threonine in Pasankalla (chemical score = 0.61) and Kurmi (chemical score = 0.94) varieties and lysine in Rosada (chemical score = 0.78) and Blanca (chemical score = 0.76) varieties, although its content was superior to the content in wheat (1.68 g of lysine/100 g of protein; Stikic et al. 2012). The varieties from Bolivia (Jacha and Chucapaca) did not present a limiting amino acid and had an excellent and balanced amino acid composition.

In agreement with results presented by Abugoch et al. (2008) for a quinoa variety from Chile, only lysine was a limiting amino acid for two- to five-year-old infants or children.

The ratio between essential amino acid and total amino acid (E/TN) of protein isolates was over 0.4 in the six quinoa varieties; therefore, proteins can be considered high quality, according to the minimum E/TN ratio of 0.36 proposed by FAO/WHO/UNU (1985).

Color. Protein isolates presented similar values of L^*, a^*, and b^* ($L^* \approx 65.9$, $a^* \approx 1.5$, and $b^* \approx 17.3$); however, the varieties from Bolivia were slightly darker and less yellow than those from Peru (Table I).

The color of isolated protein promotes its use mainly in chocolate desserts, bakery products, pasta, sausages, breakfast cereal, seasonings, and food products for children.

In comparison with amaranth and soy protein isolates (Marcone and Kakuda 1999), QPI presented a lower luminosity (L^*); this result may be owing to pigment coextraction during isoelectric precipitation of proteins.

Electrophoretic Pattern. Figure 1 shows the electrophoretic patterns of total protein extraction in reducing conditions from flour and protein isolates. The electrophoretic profiles of flour protein from quinoa varieties showed similar protein bands, except for Blanca variety, which presented two bands of approximately 50,000–60,000 and one band of 32,000, corresponding to acid subunit which forms part of 11S proteins, whereas the basic subunit was not displayed in the electrophoretic profile. However, the profile presented bands at the end of the gel corresponding to the 2S protein fraction reported by Brinegar et al. (1996) with molecular mass of 8,000–9,000.

The Rosada and Pasankalla varieties presented electrophoretic profiles similar to varieties from Bolivia (Kurmi, Jacha, and Chucapaca). These varieties presented polypeptides in the size

TABLE I
Proximate Composition of Quinoa Protein Isolates (QPI) and Color Parameters[z]

QPI	Protein (db, %)	Ash (db, %)	Carbohydrates (db, %)	L^*	a^*	b^*
Chucapaca	85.11a	1.94d	12.96c	62.78a	1.27b	12.92a
Jacha	89.85b	1.76cd	8.40b	64.11b	0.99a	14.86b
Kurmi	87.14a	1.30b	11.57c	64.63b	1.26b	16.58c
Blanca	85.22a	0.93a	13.86c	67.90c	2.24c	20.41e
Rosada	89.87b	1.48bc	8.66b	68.16c	0.90a	20.36e
Pasankalla	95.69c	2.00d	2.32a	67.85c	2.42c	18.92d

[z] Different letters in the same column indicate significant differences between samples ($P < 0.05$); $n = 3$. Carbohydrates calculated by difference. Jacha = Jacha Grano; Blanca = Blanca de Hualhuas; Rosada = Rosada de Huancayo; and db = dry basis.

TABLE II
Essential Amino Acid Composition of Quinoa Protein Isolates and Wheat Flour, and FAO Suggested Intake[w]

Essential Amino Acid	Contents (g/100 g of Protein)							FAO Suggested Intake		
	Chucapaca	Jacha	Kurmi	Blanca	Rosada	Pasankalla	Wheat Flour	2–5 Years	10–12 Years	Adult
Isoleucine	4.67d	4.32c	3.83b	3.49a	3.43a	3.50a	9.90	2.8	2.8	1.3
Leucine	5.76c	6.41d	5.82c	5.28b	4.57a	5.10b	2.65	6.6	4.4	1.9
Lysine	4.72b	4.62b	4.18b	3.36a	3.43a	3.20a	1.68	5.8	4.4	1.6
Met+Cys[x]	9.32ab	10.06ab	8.00ab	7.97ab	7.20a	10.4b	1.50[y]	2.5	2.2	1.7
Phen+Tyr[z]	7.21e	6.67d	6.00c	5.94c	4.75a	5.48b	15.92	6.3	2.2	1.9
Threonine	3.16c	3.10c	2.64bc	2.23ab	2.49abc	1.70a	2.21	3.4	2.8	0.9
Valine	3.83c	4.51e	4.02d	3.79c	3.52a	3.69b	4.16	3.5	2.5	1.3
Classification										
Hydrophobic	49.76b	62.98c	37.69a	43.56ab	37.09a	43.52ab				
Polar	47.19c	41.89abc	44.27bc	36.35a	36.00a	37.50ab				
Acid	21.80b	22.50b	22.80ab	16.75a	17.10a	19.00ab				
Basic	15.43c	13.15b	15.05c	12.28ab	12.30ab	10.80a				

[w] Wheat flour data from Stikic et al. (2012). FAO suggested intake from FAO/WHO/UNU (1985). Different letters in the same row indicate significant differences between samples ($P < 0.05$); $n = 3$. Jacha = Jacha Grano; Blanca = Blanca de Hualhuas; and Rosada = Rosada de Huancayo.

[x] Sulfur-containing amino acids: methionine (Met) and cystine (Cys).

[y] Only methionine content.

[z] Aromatic amino acids: phenylalanine (Phen) and tyrosine (Tyr).

ranges 30,000–40,000 (acidic subunits) and 20,000–25,000 (basic subunits), which in the native protein are joined by disulfide bonds, forming 11S-type proteins. Besides, these samples showed some bands of 50,000–60,000, as well as 8,000–9,000 at the end of gel bands.

The electrophoretic patterns of total flour proteins revealed a greater number of bands compared with QPI patterns, so some proteins were not extracted with the isoelectric point method, although most were isolated.

The protein isolates presented polypeptides that have estimated MWs of 50,000, 38,000, 32,000, 25,000, and 20,000. The Blanca variety had only light bands of 32,000, 25,000, and 20,000 and most of the low-MW bands compared with protein isolates from other quinoa varieties. The isolates from Rosada and Pasankalla presented an additional polypeptide band of approximately 55,000 that was not observed in protein isolates from Bolivia.

Functional Properties of Protein Isolates. Proteins play an important role in the structural and sensory properties of food products; during food production, processing, packaging, transport, storage, and consumption, the physical and chemical characteristics of proteins contribute to determine food properties. However, in their native state, proteins have a structure and conformation according to their biological function; this structure can be modified during processing. Therefore, the study of the functional properties of isolated proteins may lead to future applications in different food matrices.

Protein Solubility. Protein solubility strongly depends on pH, temperature, ionic strength, and concentration. Near the isoelectric point (pI), proteins are least soluble because the net overall charge is close to zero, and attraction between oppositely charged parts of different protein molecules occurs (Luyten et al. 2004).

The solubility of QPIs at different pHs is shown in Figure 2. It is widely known that pH variations modify protein charge distribution and, consequently, protein interactions with solvent increase or decrease. Overall, the solubility profile curves of protein are U-shaped and have a solubility minimum in the proximity of the isoelectric point.

The net positive and negative charges acquired by proteins at highly acidic and alkaline regions, respectively, promote intermolecular repulsion and thus increase the solubility (Adebowale and Lawal 2004). In this case, the solubility curve of QPIs showed lower solubility at pH 3 and 5; solubility then increased significantly at neutral and basic pH. This solubility dependence on pH was also observed by other authors for proteins from quinoa (Abugoch et al. 2008), cashew nut (Yuliana et al. 2014), and *Canavalia* (Seena and Sridhar 2005).

Kurmi, Rosada, and Pasankalla varieties presented significantly lower solubility at pH 3 and 5 (<14%) compared with Chucapaca, Jacha, and Blanca protein isolates.

The QPIs of Chucapaca had the highest solubility at acid and neutral pH with respect to other quinoa varieties; however, Blanca variety presented the highest solubility at basic pH. Overall, Pasankalla protein isolates presented low solubility at all the pH values studied. There were no trends on protein solubility with respect to variety origin or with regard to their classification of sweet or bitter.

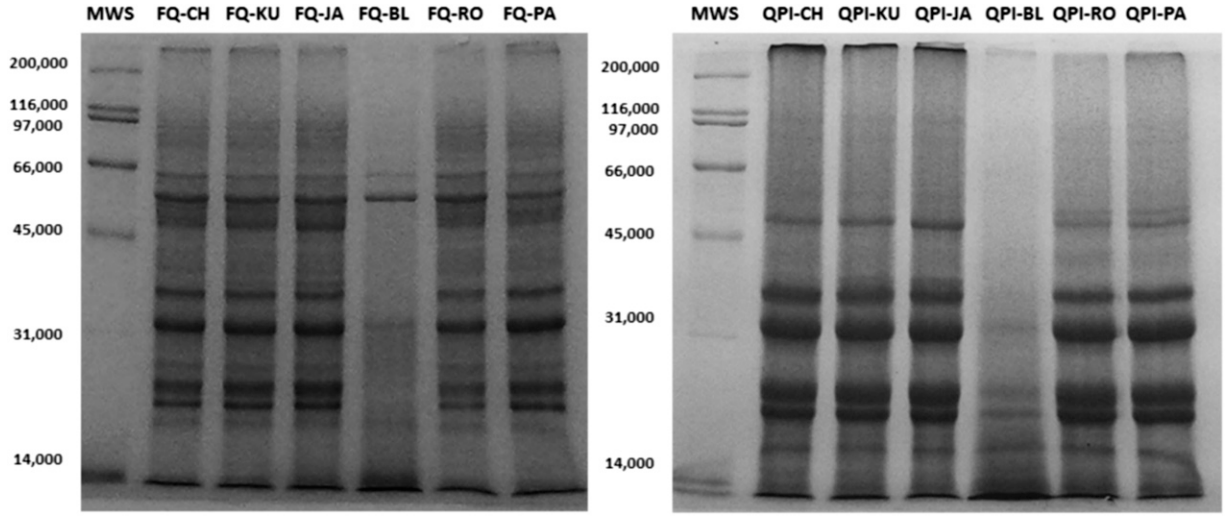

Fig. 1. Electrophoretic pattern of flour and protein isolates from quinoa (n = 3). MWS = molecular weight standard; FQ = quinoa flour; QPI = quinoa protein isolate; CH = Chucapaca; KU = Kurmi; JA = Jacha Grano; BL = Blanca de Hualhuas; RO = Rosada de Huancayo; and PA = Pasankalla.

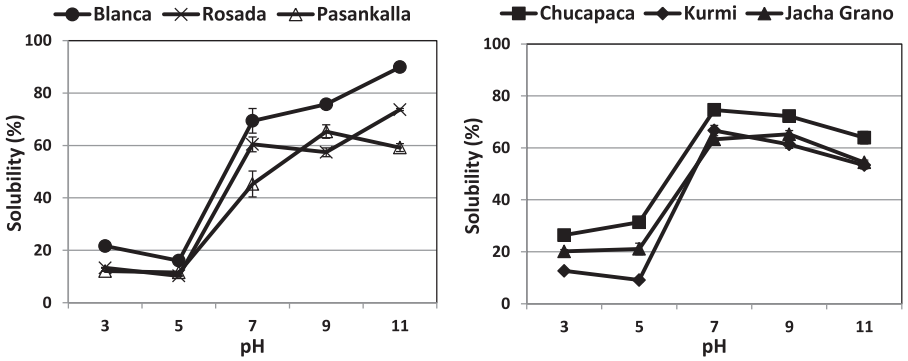

Fig. 2. Solubility at different pH values of quinoa protein isolates (n = 3). Blanca = Blanca de Hualhuas, and Rosada = Rosada de Huancayo.

Foaming Capacity and Stability. Proteins are often used in food systems to stabilize the coexistence of two or more phases. Foams, for example, consist of a water phase with air bubbles that will spontaneously separate when no stabilizer is added. The role of proteins that act as surfactants is to prevent the separation process and to retain fine air bubbles (Dickinson 1992; Tornberg et al. 1997). Food foams with varying densities can be produced in bakery products (cakes, biscuits, and sponges), beverages (beer and soft drinks), desserts (mousse and instant puddings), confectionery (marshmallows and meringues), whipped topping, and ice cream.

The foaming capacity and stability of protein isolates depended on pH and were affected by quinoa genotypes (Table III). Protein isolates had minimal foaming capacity at pH 5 (with the exception of Rosada variety), because at pH 5 proteins were near their isoelectric point and tended to agglomerate instead of interact with the aqueous medium. At acid and neutral pH, Blanca variety presented the highest foaming capacity with respect to other varieties, whereas greater foaming was observed at pH 9 in Pasankalla variety and at pH 11 in Chucapaca variety.

The highest foaming capacity at extreme pH values has been attributed to the increased flexibility of proteins, which diffuse more rapidly to the air–water interface to encapsulate air particles and enhance foaming (Aluko and Yada 1995).

Varieties that presented the greatest foaming capacities were not necessarily the most stable ones. In the case of Blanca variety, the QPI presented low-stability foam at those pH values at which it had greater foaming capacity. In general, Chucapaca, Kurmi, Jacha, and Pasankalla protein isolates presented greater foaming stability at the pH values studied, compared with Rosada and Blanca protein isolates. The flexibility and elasticity of protein interfacial films in foams depended on the nature, structure, and composition of amino acid proteins (Rouimi et al. 2005).

Quinoa proteins are mainly made of globular structure proteins; therefore, the different foaming capacity and stability of each variety could be explained in terms of the different amino acid composition and the relationship between polar and nonpolar regions of peptides.

WBC. In this work, QPIs had various WBCs; this property depended on the pH value (Table IV). All QPIs had minimum WBC at pH 5, near the isoelectric point, but this capacity increased significantly at higher or lower pH values. Similar results were observed in soy protein isolates (Elizalde et al. 1996), but Abugoch et al. (2008) did not observe significant differences in the WBC of QPIs with respect to pH values. Quinoa varieties from Peru presented higher WBC at pH >7 than quinoa from Bolivia, whereas at pH <7 protein samples showed the opposite behavior.

OBC. The three varieties from Bolivia had a significantly greater OBC compared with varieties from Peru (Table IV). The difference in the OBC may be owing to the variation in the presence of nonpolar side chains, which bind oil hydrocarbon chains through hydrophobic interactions. According to Kinsella (1976), the mechanism of fat absorption has been attributed mostly to the physical entrapment of oil but also to hydrophobicity. The Chucapaca and Jacha varieties presented the highest contents of hydrophobic amino acid (Table II) and showed the highest OBC (>4.00 g of oil/g of protein isolate), comparable to protein isolates from winged bean (Okezie and Bello 1988), soy (Elizalde et al. 1996), almond (Sze-Tao and Sathe 2000), and cashew nut (Yuliana et al. 2014).

Protein OBC depends on the nonpolar residues of the amino acids that constitute it, and this capacity is related to the behavior that proteins will have during the formation of emulsions. Nonpolar residues of proteins are primarily responsible for hydrophobic interactions with lipid molecules at oil–water interfaces of emulsions (Howell et al. 2001), although electrostatic, covalent, hydrogen, and hydrophobic forces may all contribute to protein–lipid interactions (Alzagtat and Alli 2002).

Thermal Stability. The temperature and denaturation enthalpy of protein isolates are shown in Table IV. All QPI presented endotherms corresponding to protein denaturation. The protein isolates from Bolivia presented larger denaturation enthalpy, onset temperature, and peak temperature compared with protein from Peru. This result indicates that their molecules are more structured, probably containing a higher number of hydrogen bonds (Ventureira et al. 2012). The high denaturation temperature of quinoa proteins was comparable to globulins and other cereal and legume proteins such as pea, bean, oat, and soy proteins (Arntfield and Murray 1981; Castellani et al. 1998; Meng and Ma 2001; Abugoch et al. 2008). The lower denaturation enthalpy of Pasankalla, Rosada, and Blanca

TABLE III
Foaming Capacity and Stability at Different pH Values of Quinoa Protein Isolates (QPI)[z]

	Foaming Capacity (%)					Foaming Stability (%)				
QPI	pH 3	pH 5	pH 7	pH 9	pH 11	pH 3	pH 5	pH 7	pH 9	pH 11
Chucapaca	78.6b	53.3b	107.1c	130.0c	166.7b	45.5c	93.8d	88.4d	84.6de	76.0d
Jacha	55.4a	43.3a	106.7c	96.7a	100.5a	75.3de	89.3d	87.5d	72.4bc	77.5d
Kurmi	50.0a	50.0ab	98.3c	126.7c	113.3a	67.0d	70.1c	67.8c	79.0cd	70.6c
Blanca	137.9d	83.3d	196.7d	126.7c	110.0a	34.8b	36.2a	39.0a	63.2b	57.5a
Rosada	50.0a	73.3c	58.3a	116.7b	150.0b	0.0a	45.5ab	54.3b	34.5a	66.7b
Pasankalla	100.0c	53.3b	73.3b	146.7d	103.3a	80.0e	62.5bc	100.0e	90.9e	100.0e

[z] Different letters in the same column indicate significant differences between samples ($P < 0.05$); $n = 3$. Jacha = Jacha Grano; Blanca = Blanca de Hualhuas; and Rosada = Rosada de Huancayo.

TABLE IV
Water Binding Capacity (WBC), Oil Binding Capacity (OBC), and Thermal Properties of Quinoa Protein Isolates (QPI)[z]

	WBC (g of water/g of QPI)								
QPI	pH 3	pH 5	pH 7	pH 9	pH 11	OBC (g of oil/g of QPI)	T_o (°C)	T_p (°C)	ΔH (J/g)
Chucapaca	6.18d	3.16e	4.51ab	4.50	4.13a	4.01d	93.87d	99.18b	5.35c
Jacha	4.73c	3.35de	4.31ab	4.23	4.00a	4.42e	93.37d	99.06b	5.86c
Kurmi	4.94c	2.94d	3.96a	4.53	3.93a	3.47c	92.75cd	98.38b	7.89d
Blanca	2.06a	0.81a	5.92c	6.10	10.86c	3.27b	88.02a	96.00a	2.01a
Rosada	3.04b	1.35b	5.40bc	5.31	8.18b	2.89a	89.86ab	97.99b	3.72b
Pasankalla	3.38b	1.84c	3.79a	6.67	7.36b	2.81a	91.25bc	98.59b	2.54a

[z] Different letters in the same column indicate significant differences between samples ($P < 0.05$); $n = 3$. T_o = onset temperature; T_p = denaturation temperature; ΔH = denaturation enthalpy; Jacha = Jacha Grano; Blanca = Blanca de Hualhuas; and Rosada = Rosada de Huancayo.

proteins suggested that some molecules with a small degree of folding were present.

Cluster Analysis. With the objective of determining if the differences in the physicochemical and functional properties of protein isolates depend mainly on variety origin and amino acid composition, a cluster analysis arbitrarily set to two clusters (based on the two origins of the samples) was carried out. The analysis presented a cophenetic correlation of 0.74, and the dendrogram showed two clusters: cluster 1 with three varieties from Peru (Blanca, Rosada, and Pasankalla) and cluster 2 with three varieties from Bolivia (Chucapaca, Jacha, and Kurmi) (Fig. 3). Analysis of variance indicated that clusters differed in L^* value, thermal stability, OBC, and WBC at pH 3, 5, 9, and 11; solubility at pH 11; and foaming capacity at pH 5 (Table V). The solubility and foaming capacity at pH 3, 7, and 9 did not show significant differences between the clusters. Cluster 1 with samples from Peru was typified as having the highest L^* value, the highest WBC at basic pH, and the highest foaming capacity at pH 5. Cluster 2 with samples from Bolivia was characterized as having a relatively good OBC, thermal stability, and WBC at acid pH. These data indicate that the variety origin and amino acid composition influence the functional properties of QPI despite the similarities of electrophoretic patterns.

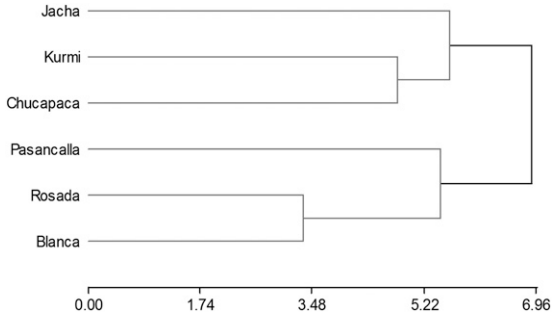

Fig. 3. Dendrogram classification of quinoa protein isolates with the method used (cluster analysis) (n = 3). Cluster 1: Blanca de Hualhuas, Rosada de Huancayo, and Pasankalla varieties. Cluster 2: Chucapaca, Jacha Grano, and Kurmi varieties.

TABLE V
Cluster Analysis of Six Quinoa Protein Isolates (QPI) Based on Different Variables[z]

Variable	Cluster 1	Cluster 2
L^*	67.97b	63.84a
OBC (g of oil/g of QPI)	2.99a	3.89b
T_p (°C)	97.53a	98.83b
ΔH (J/g)	2.76a	6.49b
WBC, pH 3	2.82a	5.28b
WBC, pH 5	1.33a	3.15b
WBC, pH 7	5.04a	4.26a
WBC, pH 9	6.03b	4.42a
WBC, pH 11	8.80b	4.02a
Solubility, pH 3 (%)	15.69a	19.72a
Solubility, pH 5 (%)	12.62a	20.52a
Solubility, pH 7 (%)	58.36a	68.19a
Solubility, pH 9 (%)	66.14a	66.27a
Solubility, pH 11 (%)	74.24b	57.24a
Foaming capacity, pH 3	95.95a	61.31a
Foaming capacity, pH 5	70.00b	48.89a
Foaming capacity, pH 7	109.44a	104.05a
Foaming capacity, pH 9	130.00a	117.78a
Foaming capacity, pH 11	121.11a	126.83a

[z] Different letters in the same row indicate significant differences between samples ($P < 0.05$); n = 3. Mean variable of each cluster analyzed by analysis of variance. Cluster 1: QPI of Blanca de Hualhuas, Rosada de Huancayo, and Pasankalla varieties. Cluster 2: QPI of Chucapaca, Jacha Grano, and Kurmi varieties. OBC = oil binding capacity; WBC = water binding capacity (g of water/g of QPI); and foaming capacity is measured in percent (%).

However, there exists a broad range of solubility and foaming capacity of proteins, regardless of their origins.

CONCLUSIONS

QPIs with high protein content were obtained by isoelectric precipitation. Electrophoresis showed that most of the protein subunits were present in the isolates. All protein isolates had a high content of essential amino acids. The lysine content was greater than for most of the cereals, and Chucapaca and Jacha grain varieties did not have limiting amino acids.

On the other hand, the six QPIs had good functional properties that make them suitable for a wide range of food applications. Through cluster analysis it was demonstrated that variety sources have an effect on the nutritional quality and functional properties of protein. The Bolivian varieties had a significantly higher content of lysine and better thermal stability, OBC, and WBC at acid pH, whereas the Peruvian varieties were characterized as having a high WBC at basic pH and a high foaming capacity at pH 5. In conclusion, QPI presented a high potential as a vegetable protein alternative for food applications, especially for vegetarian and vegan diets.

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