

Black, Q12, and Titicaca Quinoa Protein Isolate: Nutritional and Physicochemical Properties

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ABSTRACT

Quinoa is a pseudocereal plant that has been cultivated in Iran recently. The purpose of this research was to evaluate its properties for use in food. Quinoa Protein Isolates (QPIs) were obtained from Iranian Quinoa Seed cultivar (QS) varieties (Black-QS, Q12-QS, and Titicaca-QS). The Black-QPI and Titicaca (T)-QPI had a higher protein content: 87.30 ± 1.96 and $87.80 \pm 1.61\%$ w/w, respectively. The results showed foaming capacity (40.54%), stability (65.26% in 60 min), and oil absorption (3.02 mL g^{-1}) were significantly ($P \leq 0.05$) higher in Black-QPI. Textural parameters revealed that viscosity and shear stress were higher in Q12-QS than others. The amino acid profile showed that T-QS had a well-balanced profile with the highest content of tryptophan (8.23%). Consequently, the suitable nutritional and functional properties of *Titicaca* protein make it an appropriate candidate for use as a safe food additive.

Keywords: *Chenopodium quinoa* Willd., Titicaca properties.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a gluten-free dicotyledonous pseudo-grain, and is consumed by people living in the Andean Region for a very long time. There has been a growing concern about plant-based diets, applied as an alternative protein source. Recently, plant proteins are introduced as proper alternatives to animal-based ones, due to their lower side effects as compared to those associated with the consumption of animal-based proteins (Alrosan *et al.*, 2022). Moreover, gluten-free pseudocereals (Amaranth, Buckwheat, and Quinoa) exist in human diets to have outstanding nutritional value. In addition, the potential health benefits of pseudocereals have been recently pointed out as important sources for the development of functional food research. The amino-acid composition

and bioavailability of crops' proteins are important factors to examine the quality of these protein sources (Martínez-Villaluenga *et al.*, 2020). The biological value of quinoa's dietary value (73%) is nearly comparable to beef (74%). The daily consumption of quinoa is suggested to patients suffering from Cardiovascular Diseases (CVDs), high blood cholesterol and glucose, plasma antioxidant activity, and systemic inflammation (Shahbaz *et al.*, 2023).

Due to its high protein content, quinoa is considered a good source of methionine (3.6%), histidine (2.9%), and lysine (5.4%), which currently attract worldwide attention. Protein isolate is the most refined, which constructed $90 \text{ g } 100 \text{ g}^{-1}$ of the total protein of quinoa (Gupta *et al.*, 2021). Although the proteins of these important pseudo-grains are rich in essential amino acids, their poor

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functional properties including solubility, foaming water binding, and emulsifying have been approved (Mir *et al.*, 2021). An 11S globulin called chenopod is predominantly present in the mature quinoa seed. Chenopod consists of approximately 37% total protein and 2S albumin, which are stabilized by disulfide bonds. In addition, quinoa seeds contain a low concentration of Prolamins (0.57% of the total protein), which makes them suitable for celiac patients (Dakhili *et al.*, 2019). The use of protein isolation has increased due to different factors, including bioactive components, good functionality, higher levels of proteins in the food industry, and lower content of anti-nutritional factors. The alkaline pH (8-11) is one of the most effective ways to obtain protein, while for the isoelectric precipitation of solubilizing proteins an acidic pH (4-6) is applied (Abugoch *et al.*, 2008; Vega-Gálvez *et al.*, 2010).

Research on the nutritional properties of quinoa grown in Iran is limited. For instance, the amounts of available carbohydrates, fat, protein, ash, and dry matter were reported as follows: 73.14 ± 1.59 , 6.09 ± 0.30 , 16.30 ± 1.52 , 4.43 ± 0.47 , and $90.30 \pm 0.89\%$, respectively. Analysis of the amino acid profile of quinoa revealed the highest levels of lysine (3.08%) and glutamic acid (1.230%). Linoleic acid content is 63.5% in fat (Sekhavatizadeh *et al.*, 2021).

Quinoa Protein Isolate (QPI) is an impressive and promising source of nutrient that makes it a suitable nutritional supplement for functional foods. The physicochemical properties of quinoa proteins isolated from other countries have been already determined, but proteins from Iranian quinoa varieties have not been described. Hence, systematic information about the functional, chemical, and physical properties of proteins is necessary to categorize their feasible application without compromising nutritional and health-related issues. While a few studies have already investigated the quinoa proteins, there is an

urgent need to further characterize the grains, flours, and protein isolates from Black quinoa grains (Ghumman *et al.*, 2021).

This study provides a comprehensive comparative analysis of three distinct quinoa varieties—Titicaca (T-QPI), Q12 (Q12-QPI), and Black (Black-QPI), which have not been thoroughly investigated in terms of their proximate composition and functional properties in prior literature. Our research provides a systematic evaluation of the chemical, nutritional, and functional characteristics of protein isolates derived from these specific genotypes, thereby contributing to the understanding of how genetic variability influences quality, and functionality of plant-based proteins.

MATERIALS AND METHODS

Materials

Methanol, sodium hydroxide, sulfuric acid, KH_2PO_4 , NaOH, hydrochloric acid, hexane, chloroform, standards including sodium acetate, boric acid, borate buffer, methyl red, methanol (HPLC grade) hydrochloric acid, and the additional standard reagents were purchased from Merck (Darmstadt, Germany). O-phthalaldehyde, 2-Mercaptoethanol, norvalin, and pepsin were obtained from Sigma Chemical Co (St. Louis, MO, USA).

Collection and Further Identification of Seeds

The three dried genera of quinoa (saponin-free) consisting of Black-QS, T-QS, and Q12-QS were harvested from growing plants at Zarghan Station, Zarghan City, Fars Province (southern Iran) (Figures 1-A, -B, and -C). Further identification of the plant was completed by the Fars Research Center for Agriculture and Natural Resources (FRCANR), Herbarium in Shiraz, Iran. A

representative sample was finally deposited in the FRCANR herbarium, Shiraz, Iran.

Flour Preparation

The procedure for washing the seeds involved washing them four or five times with cold water, or until no foam remained to eliminate the saponins, which were then dried in the oven at $45 \pm 1.0^\circ\text{C}$ for 24 hours. Moulinex Miller (Model dePOSE 00022, France) was applied to flour the seeds, which was filtered through a 60-mesh sieve (US standard sieve), packed in polyethylene bags followed by storing at 5°C (James, 2009).

Preparation of Quinoa Protein Isolated (QPI)

For his purpose, chloroform: methanol (2:1), 1:10 w/v shaking for 2 hours was used to eliminate lipids from the quinoa flour. The procedure was repeated in triplicate. Briefly, 50 g of fat-free quinoa flour was dissolved in 1,000 mL of Milli-Q water (1:20 w/v). The pH was then adjusted to 11 using 0.1N NaOH. The maximum degree of solubilization was obtained by holding the sample in a fixed position after stirring the suspension for 24 hours. The mixture was centrifuged at $6,000 \times g$ for 30 minutes at 20°C in a refrigerated high-speed centrifuge (Sigma 3-16pk, Sigma, Osterode, Germany). Furthermore, the pH of the supernatant was adjusted to 4.5 using 0.1N HCl. The

suspension was centrifuged at $10,000 \times g$ for 45 minutes at 4°C , followed by washing three times with deionized water. The precipitate was then lyophilized, and stored at -20°C for further use (Elsouhaimy *et al.*, 2015)

Proximate Analysis of Quinoa Seeds and QPI

The crude ash, total solids, crude protein, and crude fat content of QPI and seeds were determined by using the methods of Sekhavatizadeh *et al.* (2021). The Kjeldahl method with a conversion factor of 5.85 was used to determine the crude protein content of the seeds and QPIs. Crude fat was determined by extracting a known sample aliquot with hexane using a Soxhlet apparatus. The difference in the values was used to calculate the total carbohydrates, which were presented as a percentage (Marmouzi *et al.*, 2015). The ash content of each sample was determined at $550 \pm 15^\circ\text{C}$. Energetic values and total carbohydrates were evaluated based on the following equations:

$$\text{Energy (kcal } 100 \text{ g}^{-1}) = 9 \times (m_{\text{fat}}) + 4 \times (m_{\text{carbohydrates}} + m_{\text{proteins}}) \quad (1)$$

$$\text{Total carbohydrates (g } 100 \text{ g}^{-1}) = 100 - (m_{\text{ash}} + m_{\text{proteins}} + m_{\text{fat}}), \text{ (Sekhavatizadeh } et al., 2021) \quad (2)$$

All unites are w/w.

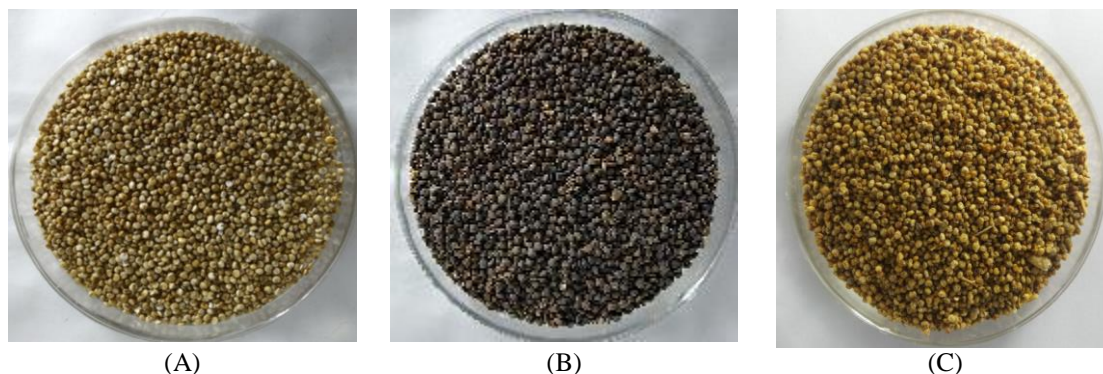


Figure 1. Quinoa Seeds (QS) included in this study: (A) T-QS, (B) Black-QS, (C) Q12-QS.



Amino Acid Analysis of Quinoa Seed

The amino acid analysis was performed after hydrolysis of seed samples with 6 mol L⁻¹ HCl and 0.5 g L⁻¹ of β-mercaptoethanol in vacuum-sealed tubes based on Sekhavatizadeh *et al.* (2021 and 2023) methods. For lysine analysis, HPLC system an auto-sampler system (Perkin Elmer, Australia) was used. The following reagents were used: 0.01 M sodium acetate in water (mobile phase A) and methanol (mobile phase B). The content of amino acid was recorded in mg 100 g⁻¹ d⁻¹ m⁻¹. For tryptophan determination, samples were decolourised with half-saturated n-butanol solution and digested in 75 mmol L⁻¹ KOH containing 0.5 g L⁻¹ β-mercaptoethanol at 110°C for 24 hours, in screw-capped test tubes. After centrifugation at 6,000×g for 30 minutes, the resulting supernatants were used for colorimetric tryptophan determination. The concentration of amino acids was expressed as g 100 g⁻¹ protein (Gonzalez *et al.*, 2012).

Foaming Capacity and Stability

The foam properties of protein isolates were determined as was described by Panozzo *et al.* (2014). For this purpose, foams were obtained by whipping 5 mL of QPIs for 3 min at 20°C in a 50 mL cylinder by a high speed mixer (ultra-turrax (IKA, T25, Staufen, Germany) operating at 9500 rpm. The volume of the foam and of the drained liquid was assessed just after whipping and during holding up to 30 minutes at 20°C. Percentage Foam Capacity (FC) (foam ability) and Stability (FS) were calculated as follows:

$$FC\% = (V_f - V_0) / V_0 \times 100 \quad (3)$$

$$FS\% = V_{f30} / V_f \times 100 \quad (4)$$

Where, V_f is the foam Volume, V_0 is the initial Volume of the QPIs and V_{f30} is the foam Volume after 30 minutes observation.

Viscosity

The viscosity of QPI samples (10%, w/v) was assessed, using a rheometer (MCR 302,

Anton Paar, Austria) (MCR 302, Anton Paar, Austria). The sample was left before the measurement of viscosity for 12 hours. The sample volume of QPI in concentric cylinder geometry was 5 mL at a temperature of 23°C and a shear rate from 10 to 100 s⁻¹ (Shaviklo *et al.*, 2012).

Oil and Water Absorption of QPIs

One gram of QPI samples was thoroughly mixed with distilled water (10 mL) for 30 seconds with a homogenizer (UltraTurrax IKA, T25, Werke, Germany). To settle the protein suspension, it was left at 25±1°C for 0.5 hour. It was centrifuged at 7,000×g for 0.5 hour and kept in a 10 mL measuring cylinder. To work out the oil absorption of the protein, the same procedure was employed (Elsohaimy *et al.*, 2015).

Statistical Analysis

To analyze the data, one-way Analysis Of Variance (ANOVA) was used with a confidence level of 0.05 (SPSS version 21.0). Duncan's multiple ranges at a significance level of 0.05 were used to compare the mean values. All experiments were carried out in triplicate.

RESULTS

Proximate Value of QPI and QS

The proximate value of QPIs and quinoa seed flour is shown in Table 1. The three quinoa flours had a significant difference in protein, carbohydrate, ash, and energy ($P \leq 0.05$). However, no significant differences in dry matter and fat content were observed ($P > 0.05$). The protein content of Black-QS flour (16.02±0.33 g 100 g⁻¹) and T-QS (16.40±0.22 g 100 g⁻¹) did not reveal any significant differences, while, a lower protein content (14.93±0.21 g 100 g⁻¹) of Q12-QS was shown. Q12-QS flour was

higher in carbohydrates than T-QS and Black-QS. The energy values in this study were (401.21±0.81 to 410.7±0.3 kcal 100 g⁻¹ dw), higher than the average value of quinoa (331-381 kcal 100 g⁻¹) (Nowak *et al.*, 2016).

The highest ash content (2.97±0.12 g 100 g⁻¹) was detected in Black-QPI, whereas the lowest value (2.13±0.6 g 100 g⁻¹) was observed in T-QPI. The highest level of pH in the Black-QPI was (5.61±0.04). The fat content of T-QPI was (0.63±0.01 g 100 g⁻¹), which was 40% higher than that of Black-QPI. The highest carbohydrate content was reported in Q12-QPI (21.42±0.96 g 100 g⁻¹), which was 44% higher than Black-QPI. The highest level of energy was (405.0±5.4 kcal 100 g⁻¹), which was allocated to the T-QPI.

Amino Acid Analysis

The composition of amino acids and chromatograms of Black-QS, Q12-QS, and T-QS are shown in Table 2 and Figure 2, respectively. The concentration of amino acids in quinoa varieties varied: with tryptophan (6.55-8.23%), glutamic acid (0.77-1.07%), and glycine (0.25-0.46%) the predominant amino acids in all varieties. T-QS was higher in amino acids than the others. Lysine (0.3%) and threonine (0.14%) were the most important essential amino

acids of T-QS, which were found as a limited amino acid in conventional grains, for example in wheat.

Foaming Capacity and Stability

The potential of QPI as a whipping agent depends on its foaming ability and stability.

Foams improve the texture, consistency, and appearance of food. The Black-QPI showed a higher foaming capacity (65.26%±11.76) than T-QPI and Q12-QPI (Table 3). However, no significant difference was found between Black-QPI and T-QPI in foam stability.

Viscosity of QPIs

The oscillatory rheology of QPIs is shown in Figure 3. As expected, all QPI samples were characterized as Newtonian liquids. As a result, the association between shear rate and resultant stress is linear, as with Newtonian fluid. There were no significant differences among the QPI samples. In addition, the shear rate versus viscosity relationships of QPIs are shown in Figure 3-B.

Table 1. Proximate value (Mean±SD, n= 3) of three Quinoa Seeds (QSs) and Quinoa Protein Isolated (QPI) of three genera (Black, Q12, and Titicaca).^a

Parameters	Black-QS	Q12-QS	T-QS	Black-QPI	Q12-QPI	T-QPI
pH	6.74±0.04a	6.45±0.05b	6.04±0.01c	5.61±0.04C	4.48±0.03A	4.84±0.03B
Dry matter (g 100 g ⁻¹ as fed)	95.10±1.47a	95.78±1.96a	95.29±1.24a	98.20±0.15A	98.35±0.22A	98.22±0.09A
Protein (g 100 g ⁻¹ dw)	16.02±0.33a	14.93±0.21b	16.40±0.22a	81.72±1.83A	75.42±0.87B	82.18±1.51A
Fat (g 100 g ⁻¹ dw)	3.62±0.16a	3.73±0.06a	3.90±0.20a	0.45±0.5B	0.56±0.03A	0.63±0.01A
Ash (g 100 g ⁻¹ dw)	5.43±0.15a	4.36±0.22b	3.67±0.2c	2.97±0.12A	2.60±0.1B	2.13±0.6C
†Carbohydrates (g 100 g ⁻¹ dw)	74.93±0.14c	76.98±0.19a	76.03±0.34b	14.86±1.74B	21.42±0.96A	15.06±1.43B
‡Energy (kcal 100 g ⁻¹ dw)	409.6±0.7c	401.21±0.81 b	410.7±0.3a	404.4±6.6B	404.1±3.5A	405.0±5.4C

^a Values are expressed as mean±SD. Means in the same row with different lowercase letters (A–C) among quinoa protein isolate (QPI) (Black, Q12 and Titicaca) averages differ significantly (P≤ 0.05). Means in the same row with different lowercase letters (a–c) among quinoa seeds (Black, Q12, and Titicaca) averages differ significantly (P≤ 0.05). dw: Dry weight, Quinoa Protein Isolate (QPI); Quinoa Seed (QS); Titicaca (T).

† Total carbohydrate (g 100 g⁻¹)= 100-(m_{fat}+m_{ash}+m_{proteins}); ‡ Energy= 4× (%Protein+%Carbohydrates)+9×(%Fat).

Table 2. Amino acid profile in three quinoa generations (Black-QS, Q12-QS, T-QS).^a

Amino acid (g 100 g ⁻¹)	Black-QS	Q12-QS	Titicaca-QS
Aspartic acid	0.44	0.45	0.57
Glutamic acid	0.77	0.86	1.07
Serine	0.14	0.17	0.25
Tyrosine	< 0.06	< 0.06	< 0.06
Arginine	< 0.06	0.16	0.18
Methionine	< 0.06	< 0.06	0.07
Tryptophan	6.55	7.99	8.23
Valine	0.17	0.20	0.29
Isoleucine	0.03	0.09	0.17
Lysin	< 0.06	< 0.06	0.30
Phenylalanin	< 0.06	< 0.06	0.08
Leucin	0.18	0.22	0.31
Histidin	< 0.06	< 0.06	< 0.06
Glycin	0.25	0.37	0.46
Teronin	0.06	0.09	0.14
Alanin	0.14	0.17	0.3

^a Quinoa Seed (QS); Titicaca (T).

Table 3. Foaming capacity and stability (Mean±SD, n= 3) of Quinoa Protein Isolated (QPI) of the three genera (Black, Q12, and Titicaca).^a

QPI genera	Protein conc.% (w/v)	Foaming capacity (%)	Foaming stability % at time interval (min)				
			0.5	5	10	40	60
Black-QPI	0.1	50.01±1.77 ^E	75.38±3.86 ^{aE}	70.77±2.72 ^{aC}	50.77±5.44 ^{bDE}	38.46±2.68 ^{cF}	33.85±2.69 ^{cG}
	0.5	60.03±2.32 ^C	78.22±0.44 ^{aCDE}	77.02±3.82 ^{aAB}	57.63±4.12 ^{bBC}	42.23±3.03 ^{cEF}	37.20±1.33 ^{cFG}
	1	72.53±2.08 ^B	80.60±0.92 ^{aBCD}	77.49±0.91 ^{bAB}	57.51±1.06 ^{cEF}	46.26±2.17 ^{dCDE}	42.49±0.99 ^{eDE}
	3	76.93±3.36 ^A	84.50±1.64 ^{aAB}	78.49±1.87 ^{bAB}	65.68±0.36 ^{cA}	53.00±2.34 ^{dB}	49.02±0.47 ^{eAB}
	Average	65.26±11.76 ^b	79.77±3.90 ^a	76.04±3.89 ^a	55.91±7.73 ^a	45.00±6.04 ^c	40.54±6.12 ^a
Q12-QPI	0.1	43.09±3.09 ^F	67.92±1.62 ^{aF}	48.43±4.95 ^{bE}	39.38±2.75 ^{cH}	33.89±1.20 ^{dG}	28.67±2.85 ^{dH}
	0.5	49.24±2.34 ^E	70.38±2.97 ^{aF}	53.17±4.50 ^{bDE}	43.74±3.66 ^{cGH}	38.99±2.47 ^{cdF}	34.43±3.75 ^{dFG}
	1	53.09±3.49 ^{DE}	76.87±1.56 ^{aDE}	49.41±3.73 ^{bE}	41.98±1.72 ^{cFG}	39.13±1.25 ^{cdF}	36.17±1.91 ^{dFG}
	3	55.39±0.43 ^D	83.33±5.01 ^{aBCD}	58.33±1.39 ^{bD}	54.17±2.41 ^{bcCDE}	50.00±2.41 ^{cdCD}	41.67±3.67 ^{dCD}
	Average	50.20±5.35 ^a	74.28±6.30 ^b	51.99±4.84 ^b	44.77±5.76 ^b	39.81±5.25 ^b	35.58±5.99 ^b
T-QPI	0.1	50.00±0.35 ^E	78.46±1.66 ^{aCDE}	73.85±1.42 ^{bBc}	55.38±2.74 ^{cBCD}	44.62±1.47 ^{dDE}	38.46±4.13 ^{eEF}
	0.5	53.08±1.7 ^{ED}	81.11±4.16 ^{aBCD}	76.76±4.24 ^{Aab}	57.95±1.8 ^{bBC}	49.23±3.33 ^{cBC}	42.05±1.04 ^{dDE}
	1	56.17±2.74 ^D	83.60±1.78 ^{aABC}	78.11±1.62 ^{Aab}	58.86±2.85 ^{cB}	52.12±3.27 ^{dB}	46.53±1.88 ^{eBC}
	3	74.62±0.69 ^B	88.68±4.43 ^{aA}	82.49±2.63 ^{bA}	65.99±2.39 ^{cA}	58.77±1.64 ^{dA}	52.57±1.85 ^{eA}
	Average	58.47±10.10 ^a	82.96±4.83 ^a	77.80±3.99 ^a	59.55±4.62 ^a	51.18±5.8 ^a	44.91±5.90 ^a

^a Data (mean ± standard deviation) are from three replications. Quinoa protein isolate (QPI); Titicaca (T); Means in the same column with different uppercase letters (A-H) and rows with different lowercase letters (a-e) among (Q12-QPI, Black-QPI, and T-QPI) differ significantly ($P \leq 0.05$); Means in the same column and rows with different bold underline lowercase letters (a–b) among (Q12-QPI, Black-QPI, and T-QPI) averages differ significantly ($P \leq 0.05$).

that of Gómez *et al.* (2021) results that reported a protein content range of 15.59–18.73% with The observed protein content of quinoa (14.93±0.21 to 16.40±0.22% w/w) is consistent with the findings of Gómez *et al.*, who reported a protein content range of 15.59–18.73% w/w.

According to the FAO/WHO/UNU (2007) standards for protein quality, quinoa protein can provide substantial excesses of several

essential amino acids relative to the recommended levels for adult nutrition. Specifically, it supplies approximately 180% of the histidine requirement, 274% of isoleucine, 338% of lysine, 212% of methionine plus cysteine, 320% of phenylalanine plus tyrosine, 331% of threonine, 228% of tryptophan, and 323% of valine. Moreover, quinoa contains unusually high concentrations of sulfur-containing

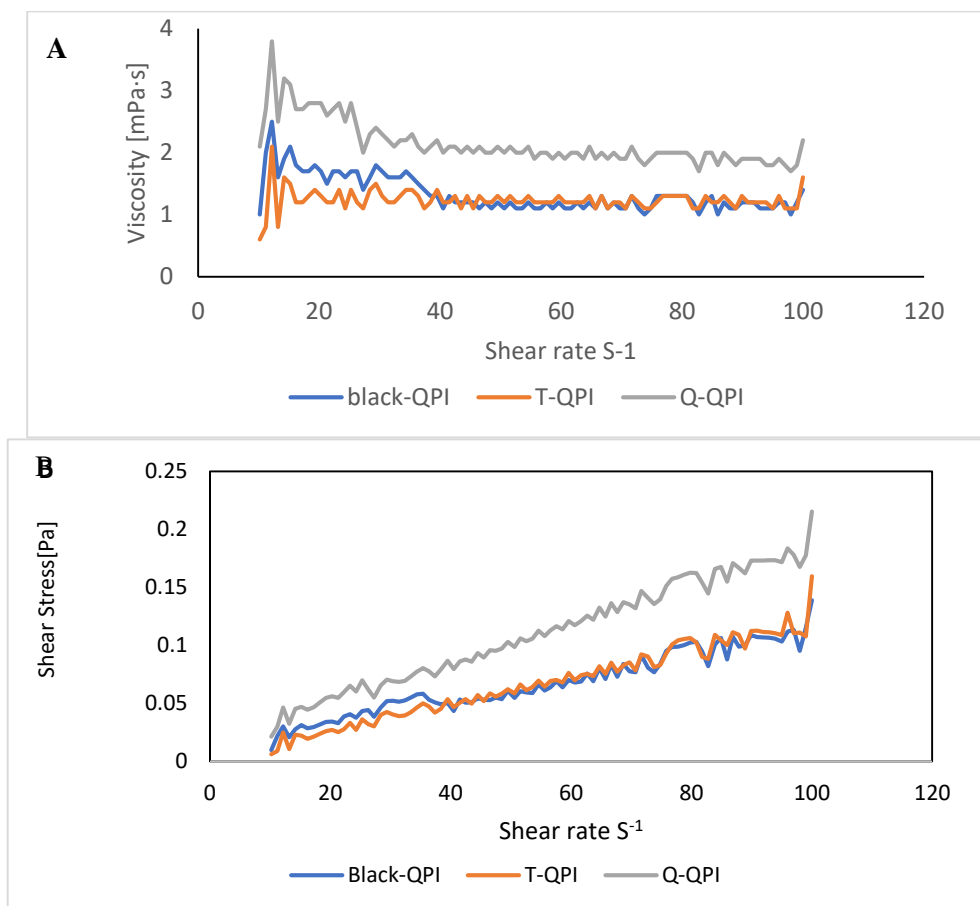


Figure 3. (A) The apparent viscosity, and (B) Shear stress versus shear rate curves of QPIs samples (Black-QPI, Q12-QPI, T-QPI). Quinoa Protein Isolate (QPI), Titicaca (T).

Table 4. Oil, and water absorption parameters (Mean±SD, n= 3) of Quinoa Protein Isolated (QPI) of the three genera (Black, Q12, and Titicaca).^a

Parameters		Black-QPI	Q12-QPI	T-QPI
Water and oil absorption	WA (mL g ⁻¹)	1.9±0.01b	1.0±0.06c	2.02±0.02a
	OA (mL g ⁻¹)	3.02±0.03a	2.0±0.02c	2.42±0.03b

^a WA: Water Absorption; OA: Oil Absorption; Quinoa protein isolate (QPI), Titicaca (T).

amino acids—methionine and cysteine—compared to most other plant sources. The overall profile of essential amino acids in quinoa surpasses that of the conventional cereal grains (Vega-Gálvez *et al.*, 2010). Histidine, isoleucine, lysine, sulfur amino acids, aromatic amino acids, threonine, tryptophan, and valine content met the daily requirements for these amino acids for all age groups (Craine *et al.*, 2020). Similarly, Dini *et al.* (1992) found that decorticated quinoa exhibited nutritional properties equal

to or better than those of commonly consumed cereals. Additionally, quinoa is recognized as an exceptional source of leaf protein concentrate, indicating its potential use as a protein substitute in both human food and animal feed, as well as in pharmaceutical applications (Vega-Gálvez *et al.*, 2010).

The carbohydrate content of quinoa (74.93±0.14 to 76.03±0.34%) was comparable to the results of Saavedra and Carmen Valdez-Arana (2021) who observed

a carbohydrate content of $70.81 \pm 0.11\%$ (Saavedra and Carmen Valdez-Arana, 2021).

Starch is the primary carbohydrate component in quinoa, accounting for 52 to 69% of its total composition. The total dietary fiber content of quinoa is comparable to that found in other cereal grains, ranging from 7 to 9.7%, with soluble fiber making up 1.3 to 6.1%. Quinoa also contains approximately 3% sugars, primarily in the form of maltose, D-galactose, and D-ribose, along with smaller amounts of fructose and glucose (James, 2009).

Due to its functional properties, quinoa serves as an effective thickening agent for sauces, soups, and flours. Its resistance to retrogradation further expands its culinary applications, enabling the creation of creamy smooth textures that mimic those of fats (Vega-Gálvez *et al.*, 2010; James, 2009).

The amylose content of quinoa starch ranges from 3 to 22%, which is lower than that of wheat and corn, higher than certain barley varieties, and comparable to common rice types. Compared to starches from wheat and barley, quinoa starch demonstrates greater maximum viscosity, enhanced water absorption capacity, and superior swelling power. Moreover, it exhibits notable stability during freezing and retrogradation processes (Tang *et al.*, 2002).

After all, the T-QS meal contained more energy than the others. The differences might be due to the interaction of various factors, including cultivars, analytical methods, and environmental conditions (Nowak *et al.*, 2016). The variations found among genera were supported by others (Alvarez-Jubete *et al.*, 2009; Nascimento *et al.*, 2014; Palombini *et al.*, 2013). These remarkable variations in the content of QPIS nutrients were noticed among different genera. The possible explanations for these variations are associated with the interaction of numerous factors including crop genetics, analytical methods, and multiple environmental circumstances (Razzeto *et al.*, 2019).

Cereals are a fundamental component of the human diet, providing approximately half of the dietary energy and protein intake for many populations. When we compare the nutritional composition of commonly consumed cereals with that of quinoa, it exhibits higher levels of protein, fat, and ash content compared to traditional cereals (Filho *et al.*, 2017).

The percentage of protein, in the current work, has been considerably improved when it was compared to the data reported by Abugoch *et al.* (2008) (77.2 and 83.5% in Q9 and Q11 QPIs, respectively) while, this was lower than the report of Ruiz *et al.* (2016) (90~93%) in sweet variety of Atlas quinoa. Such differences in the of the proteins' percentage were related to the varieties of quinoa (mentioned before), extraction, and post-extraction processes. For example, Wang *et al.* (2021) reported that QPI (Qingli 2 cultivar) and samples treated with microwave heating, steaming, boiling, and baking showed protein's contents of 89.8, 87.9, 89.1, 88.6, and 88.1%, respectively (Abugoch *et al.*, 2008; Ruiz *et al.*, 2016; Wang *et al.*, 2021). Quinoa protein isolate represents a promising nutritional ingredient with strong potential for use as a food supplement or functional food component. Beyond its high nutritional value—including a complete amino acid profile—it exhibits functional properties that make it well-suited for incorporation into cereal-based and other food products. These functional attributes, which are linked to the protein's physicochemical characteristics, play a key role in food processing and product development. As a nutrient-dense source of protein, fibre, healthy fats, and carbohydrates, quinoa can contribute meaningfully to balanced diets when consumed alongside a variety of other foods (Elsouhaimy *et al.*, 2015). Quinoa protein has gained attention as a high-quality plant-based protein due to its balanced amino acid profile, particularly its high lysine content. It exhibits good functional properties such as solubility, emulsification, and gelation,



which can be enhanced through processing techniques like fermentation and enzymatic hydrolysis. These proteins also possess antioxidant activity, contributing to food stability and health benefits. With the support of emerging green technologies, quinoa protein shows strong potential as a sustainable alternative to dairy proteins in food formulations (Alrosan *et al.*, 2022).

Amino Acid Analysis of Quinoa Seed

A wonderful amino acid profile was discovered in the quinoa seed, with acceptable amounts of Essential Amino Acids (EAAs) which are playing a crucial role in the growth and maintenance of metabolic activities and a desirable bioavailability. The QPIs are predominantly rich in histidine, methionine, and lysine that are generally observed in limited amounts in other common grains (Dakhili *et al.*, 2019).

In amino acid measurement, different findings are shown by Gómez *et al.* (2021). Different genotypes and years of growth of the plant can potentially influence these variables both in the calibration and the external validation set. This was ultimately important for developing calibration equations for future predictions (Escuredo *et al.*, 2014).

High amounts of all the essential amino acids, except methionine (0.33-0.41%), were recorded in the amino acid profiles of two pigeon pea varieties and two chickpea selections. In this work, the methionine value was lower than pigeon pea and chickpea. Lysine content was also higher (7.45–7.90%) in pigeon pea and chickpea varieties compared with QPIs (\leq 0.06-0.3%). The values of tryptophan were higher in QPIS (6.55-8.23%) than the mentioned legumes (0.46-0.96) (Anitha *et al.*, 2020).

Quinoa stands out as a highly nutritious plant-based protein source, with amino acid content closely aligned with FAO recommendations. It provides all essential amino acids, particularly rich in lysine and sulfur-containing amino acids, making its

protein quality superior to many cereal grains. Research indicates that the bioavailability of quinoa proteins improves significantly after cooking, varying depending on the variety consumed. Quinoa has high protein content and notable levels of tryptophan, often a limiting amino acid in other plants, which plays a key role in serotonin production. Additionally, quinoa contains non-protein tryptophan forms that are more readily absorbed, potentially enhancing brain function through improved neurotransmitter synthesis (Navruz-Varli *et al.*, 2016).

Foaming Capacity and Stability

The foaming properties of Quinoa Protein Isolates (QPIs) was evaluated as critical functional characteristics, particularly for their potential application in food systems requiring aeration, such as baked goods. Foaming ability generally increased with rising QPI concentration, ranging from 50.01 \pm 1.77 to 76.93 \pm 3.36% for Black-QPI, 43.09 \pm 3.09 to 55.39 \pm 0.43% for Q12-QPI, and a constant value of 50.00 \pm 0.35% for T-QPI. Among all samples, Black-QPI exhibited the highest average foaming ability (65.26 \pm 11.76%). Similarly, foaming stability improved with increasing concentration but declined over time. At 0.5 minutes of storage, foaming stability ranged from 75.38 \pm 3.86 to 84.50 \pm 1.64% for Black-QPI, 67.92 \pm 1.62 to 83.33 \pm 5.01% for Q12-QPI, and 78.46 \pm 1.66 to 88.68 \pm 4.43% for T-QPI, with T-QPI showing the highest average (82.96 \pm 4.83%). These results highlight the strong capacity of quinoa proteins to form stable foams, indicating their promising applicability in food formulations. Compared to egg albumin — a well-known excellent foaming agent with reported foaming ability values of 156–200% and foaming capacity of 33–54% (Lomakina and Mikova, 2006). Quinoa protein demonstrated relatively lower foaming ability, but comparable foam stability (35–44%). The foam stability of

QPI was found to be significantly higher than that of soybean protein, and slightly lower than that of egg white protein (Abugoch *et al.*, 2008). This behavior may be attributed to protein unfolding at low pH, which exposes hydrophobic regions and enhances interfacial activity.

Additionally, molecular configuration and solubility play crucial roles in determining foaming performance, with more flexible proteins typically exhibiting superior foaming properties (Jan *et al.*, 2018). Since foaming capacity and stability are influenced by factors such as interfacial film properties, moisture retention, and surface hydrophobicity, higher net charge can enhance solubility by reducing hydrophobic interactions and facilitating rapid spreading at the air–water interface (Ghumman *et al.*, 2021). The observed differences among QPI variants may also be related to variations in protein content and structural characteristics. For instance, Q12-QPI had the lowest protein content (Table 1), which corresponded with its inferior foaming properties. Moreover, Steffolani *et al.* (2016) emphasized that different quinoa genotypes exhibit variable foaming behaviors, underscoring the importance of genetic and compositional factors in determining functionality. Overall, these results suggest that certain QPI varieties, particularly T-QPI and Black-QPI, hold significant potential for use in aerated food products like cakes and meringues (Ogungbenle *et al.*, 2009).

Viscosity

Proteins are highly functional molecules in food systems that facilitate processing and affect the final product performance. Functional properties denote the physicochemical properties that govern protein behavior in foods with regards to their distinct amino acid sequences, molecular weight and other factors. Viscosity plays an important role that affects protein stability in food processing and

product application. Highly concentrated proteins are considered highly viscous; thus, its viscosity is considered as the most important factors to control in food processing (Yolandani *et al.*, 2023). The viscosity of plant protein dispersions is affected by factors such as pH, temperature, protein concentration, and ionic strength, making it essential to optimize these parameters for the desired consistency. One advantage of plant proteins is their ability to provide thickening and structural stability, enhancing product quality without the use of animal-derived ingredients. Their application in food formulations allows for the development of sustainable, nutritious, and texturally desirable plant-based alternatives to traditional dairy and meat products (Roy *et al.*, 2025).

Oil and Water Absorption of QPIs

The water and oil absorption of food materials is an important functional property that improves the sustainability of texture and flavour. In similar research, water and oil absorption capacities of quinoa seed were 147% and 46%, respectively (Abugoch *et al.*, 2008). Previous studies on the water and oil absorption capacity of QPIs by Ashraf *et al.* (2012) and Elsohaimy *et al.* (2015) showed that these mentioned factors had 3.94 ± 0.06 and 1.88 ± 0.02 mL g⁻¹ protein, respectively. Recently, Reséndiz *et al.* (2019) studied the oil absorption capacity of QPIs and discovered that QPIs had a 2.66 mL g⁻¹ value, which supported the results of the present work (Ashraf *et al.*, 2012; Elsohaimy *et al.*, 2015; Reséndiz *et al.*, 2019).

Oil intake is of utmost importance as oil acts as a flavor reservoir, it enhances the mouth feel of food. This indicates that Black-QPI may have stronger flavor retention than the other types. The oil and water absorption capacities were different among the genera. This can be explained by the difference between the varieties of quinoa and the areas where the quinoa



germinated (El Sohaimy *et al.*, 2018). The oil absorption capacity depends on the amount of exposed hydrophobic amino acid residues in the protein and the hydrophobic amino acid content. The water absorption rate of quinoa protein depended on the method of drying the protein and the pH level. Furthermore, this can be attributed to the particle size and larger specific surface area of QPI. We specifically highlight significant differences in protein content, carbohydrate composition, and key functional properties such as foaming capacity, water and oil absorption, and rheological behavior. Additionally, we provide detailed proximate composition data for both native seeds and isolated proteins, which can serve as a valuable reference for future studies aimed at optimizing food formulation and developing novel plant-based protein products. Overall, this study increases the scientific value of quinoa by providing a framework for selecting varieties based on specific nutritional and functional criteria for food applications.

CONCLUSIONS

In conclusion, significant differences in the chemical composition, structure, and rheological properties of Quinoa Protein Isolates (QPIs) from three varieties were identified. These were Titicaca (T-QPI), Q12 (Q12-QPI), and Black (Black-QPI), likely due to inherent seed composition differences. Q12-QPI showed the best rheological performance and suitability for texture-demanding food applications, while Black-QPI excelled in protein content, foaming, and oil absorption, making it ideal for emulsification and aeration. T-QPI demonstrated superior water absorption, beneficial for moisture retention, and showed similar protein content to Black-QPI, indicating comparable nutritional value. Titicaca quinoa seeds also exhibited the most balanced essential amino acid profile, emphasizing their potential as a high-quality plant protein. These results

highlight the importance of variety selection in optimizing quinoa proteins for specific food functions. Future research is needed to enhance processing methods that maintain protein quality across genotypes.

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خواص تغذیه ای و فیزیکیوشیمیایی ایزوله پروتئین های کینوا در ارقام سیاه، Q12 و تیتیکاکا

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چکیده

کینوا یک شبه غله است که اخیراً در ایران کشت می شود. هدف از این تحقیق بررسی خواص پروتئین ایزوله آن برای استفاده در غذا می باشد. ایزوله های پروتئین کینوا از واریته های دانه کینوا سیاه، Q12 و تیتیکاکا استخراج شدند. محتوای پروتئین کینوای سیاه و تیتیکاکا به ترتیب $(87/30 \pm 1/96)$ ، $(87/80 \pm 1/161)$ وزنی/وزنی بوده است. نتایج نشان داد در پروتئین کینوای سیاه ظرفیت کف کردن $(40/54)$ درصد، پایداری کف $(65/26)$ درصد در 60 دقیقه و جذب روغن $(3/02)$ میلی لیتر بر گرم) به طور معنی داری $(P \leq 0.05)$ بیشتر از سایر نمونه ها بود. پارامترهای بافتی نشان داد که ویسکوزیته و تنش برشی در Q12 بیشتر از سایرین بود. پروفایل اسید آمینه نشان داد که رقم تیتیکاکا دارای پروفایل متعادل با بالاترین محتوای تریپتوفان $(8/23)$ درصد بوده است. در نتیجه، ارزش غذایی و عملکردی مناسب پروتئین کینوای تیتیکاکا، آن را به عنوان گزینه مناسبی عنوان افزودنی در مواد غذایی تبدیل می کند.