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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 isolated 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, 87.80±1.61% w/w), respectively. The results showed foaming capacity (40.54%), stability (65.26% $_{\text{in 60 min}}$), and oil absorption (3.02 ml/g) were significantly ($p \leq 0.05$) was 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: Black, Q12, Quinoa Protein isolate, Titicaca.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), referred to, a gluten-free dicotyledonous pseudo-grain 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) are existing tendencies 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.,

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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 CVDs, high blood cholesterol and glucose, plasma antioxidant activity, and systemic inflammation (Shahbaz et al., 2022).

Quinoa protein, due to its high protein content is considered a good source of methionine (3.6 %), histidine (2.9 %), and lysine (5.4 %) which currently attracts worldwide attention. Protein isolate is the most refined, which constructed 90 g /100 g 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 functional properties including solubility, foaming water binding, and emulsifying have been approved (Mir et al., 2021). An 11S globulin called chenopodin is predominantly present in the mature quinoa seed. Chenopodin consists of approximately 37% total protein and 2S albumin, which are stabilized by disulfide bonds. In addition, quinoa seeds contain a low concentration of prolamines (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 and 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±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 the quality and functionality of plant-based proteins. 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.

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, norovalin, pepsin, and 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) (Figs 1A, 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 FCANR herbarium, Shiraz, Iran.

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

Flour preparation

The procedure for washing the whole 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 ± 1.0 °C for 24 h. Moulinex Miller (Model depose 00022, France) was applied to flour the seeds, the flour 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)

Chloroform: methanol (2:1), 1:10 w/v shaking for 2 h, 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 1000 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 h. The mixture was centrifuged at 6000 *g* for 30 min 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 10000 *g* for 45 min at 4 °C followed by washing three times with deionized water. The precipitate was then lyophilized, and stored at -20 °C for further use (Elsohaimy 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 total carbohydrates, which were presented as a percentage (Marmouzi et al., 2015). The ash content of each sample was determined at 550 ± 15 °C. Energetic values and total carbohydrates were evaluated based on the following equations:

Energy (kcal/100 g) =9× (m_{fat})+4×($m_{carbohydrates}$+$m_{proteins}$) (1)

Total carbohydrates (g/100 g) =100−(m_{ash}+$m_{proteins}$+m_{fat}), (Sekhavatizadeh et al., 2021) (2)

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 of β-mercaptoethanol in vacuum-sealed tubes based on Sekhavatizadeh et al., 2021 and 2023 methods. For lysin analysis HPLC system an autosampler system (Perkin Elmer, Australia) was used. 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/100g d. m. 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 h in screw-capped test tubes. After centrifugation at 6000×g for 30 min the resulting supernatants were used for colorimetric tryptophan determination. The concentration of amino acids was expressed as g/100g 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 propose, 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 min at 20 °C. Percentage foam capasity (FC) (foam ability) and stability (FS) were calculated as follows:

FC%=(Vf-V0)/V0×100 (3)

FS%=Vf30/Vf×100 (4)

Where, Vf is the foam volume, V0 is the initial volume of the QPIs and Vf30 is the foam volume after 30 min observation.

Viscosity

The QPI samples (10%, w/v), using a rheometer (MCR 302, Anton Paar, Austria). The sample was left before the measurement of viscosity for 12 h. 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 (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 s with a homogenizer (UltraTurrax IKA, T25, Werke, Germany). To settle the protein suspension, it was left at 25 ± 1 °C for 0.5 h. It was centrifuged at 7000 g for 0.5 h 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 demonstrated 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%) and T-QS (16.40±0.22%) did not reveal any significant differences, while, a lower protein content (14.93±0.21%) 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/ $_{100\,g\,d}$) , higher than the average value of quinoa (331-381 kcal/100 g) (Nowak et al., 2016).

The highest (2.97±0.12 g/100 g) and lowest ash contents were respectively detected in the Black-QPI and 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) which was 70% higher than that of Black-QPI. The highest carbohydrate content was reported in Q12-QPI (21.42±0.96 g/100 g) which was 88% higher than

that Black-QPI. The highest level of energy was (405.0±5.4 kcal/100 g) which was allocated to the T-QPI.

Table 1. Proximate value (Mean ± SD, n = 3), of three quinoa seed (QS) and quinoa protein isolated (QPI) of three genera (Black, Q12, and Titicaca).

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 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 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 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 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 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 dw)	409.6±0.7c	401.21±0.81b	410.7±0.3a	404.4±6.6B	404.1±3.5A	405.0±5.4C

Values are expressed as mean ± SD; dw: Dry weight, Quinoa protein isolate (QPI); Quinoa seed (QS); Titicaca (T); † Total carbohydrate (g/100 g) =100 − (m_{fat}+ m_{ash}+ $m_{proteins}$); ‡ Energy =4× (% protein+ %carbohydrates) + 9× (% fat); Means in the same row with different lowercase letters (a–c) among quinoa seeds (Black, Q12, and Titicaca) averages differ significantly ($P \leq 0.05$); Means in the same row with different lowercase letters (A–C) among quina protein isolate (QPI) (Black, Q12 and Titicaca) averages differ significantly ($P \leq 0.05$).

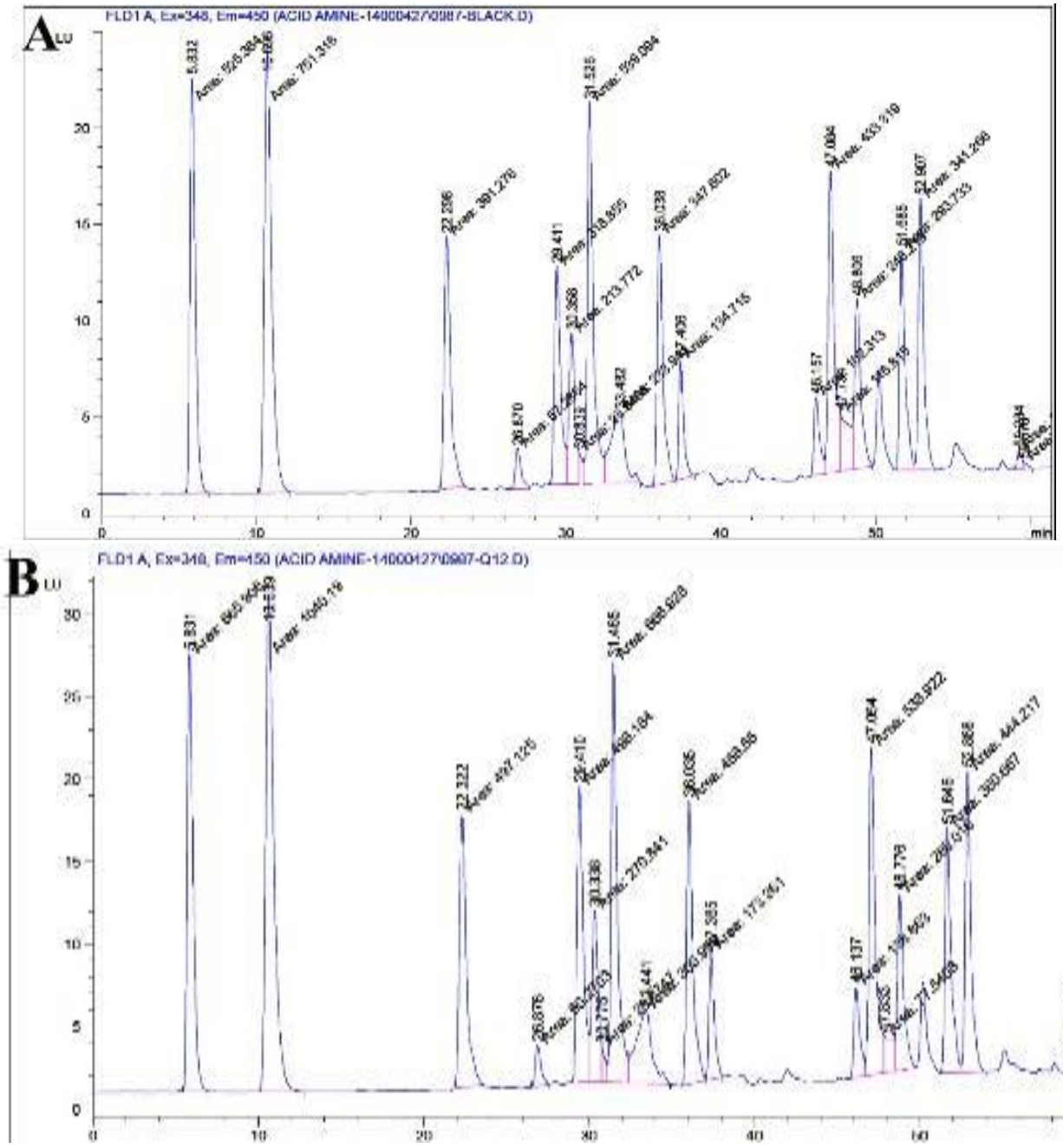
Amino acid analysis

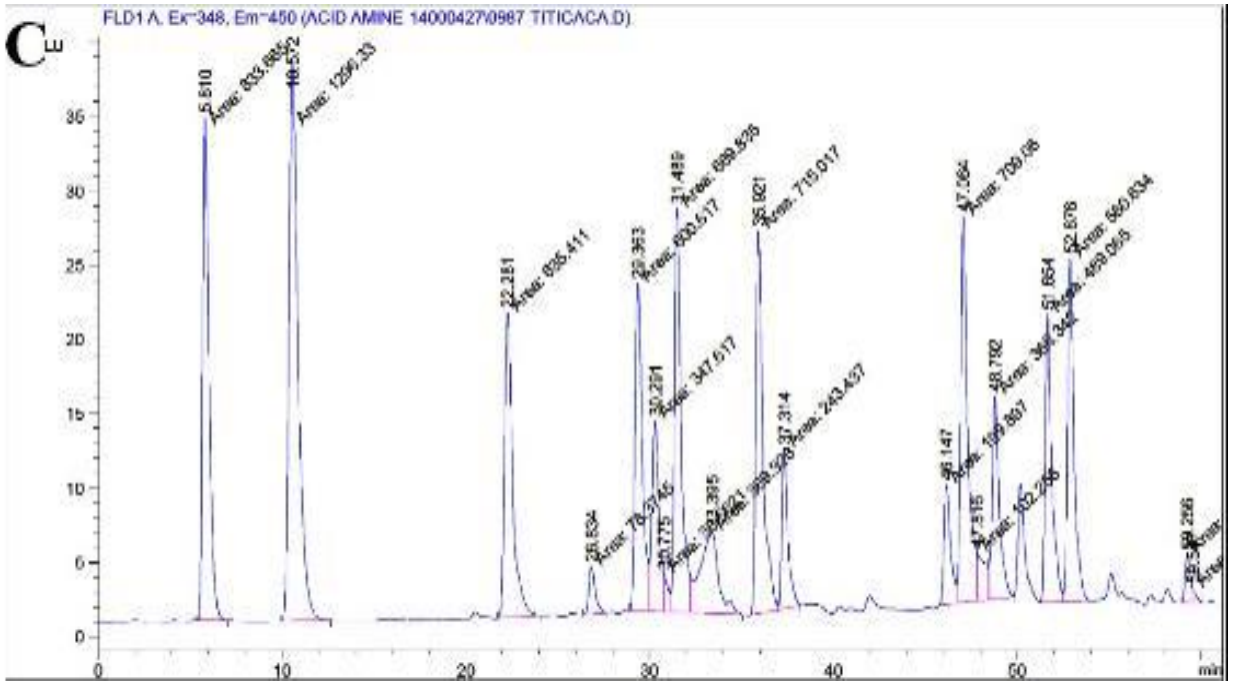
The composition of amino acids and chromatograms of Black-QS, Q12-QS, and T-QS are demonstrated in Table 2 and Fig 2, respectively. The concentration of amino acids in quinoa varieties varied, with tryptophan (6.55-8.23 %), glutamic acid (0.77-1.07 %), and glycine (0.25-0.46 %) the predominant amino 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 are found as a limited amino acid in conventional grains, for example in wheat.

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

Amino acid (g/100g)	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

Quinoa seed (QS); Titicaca (T).





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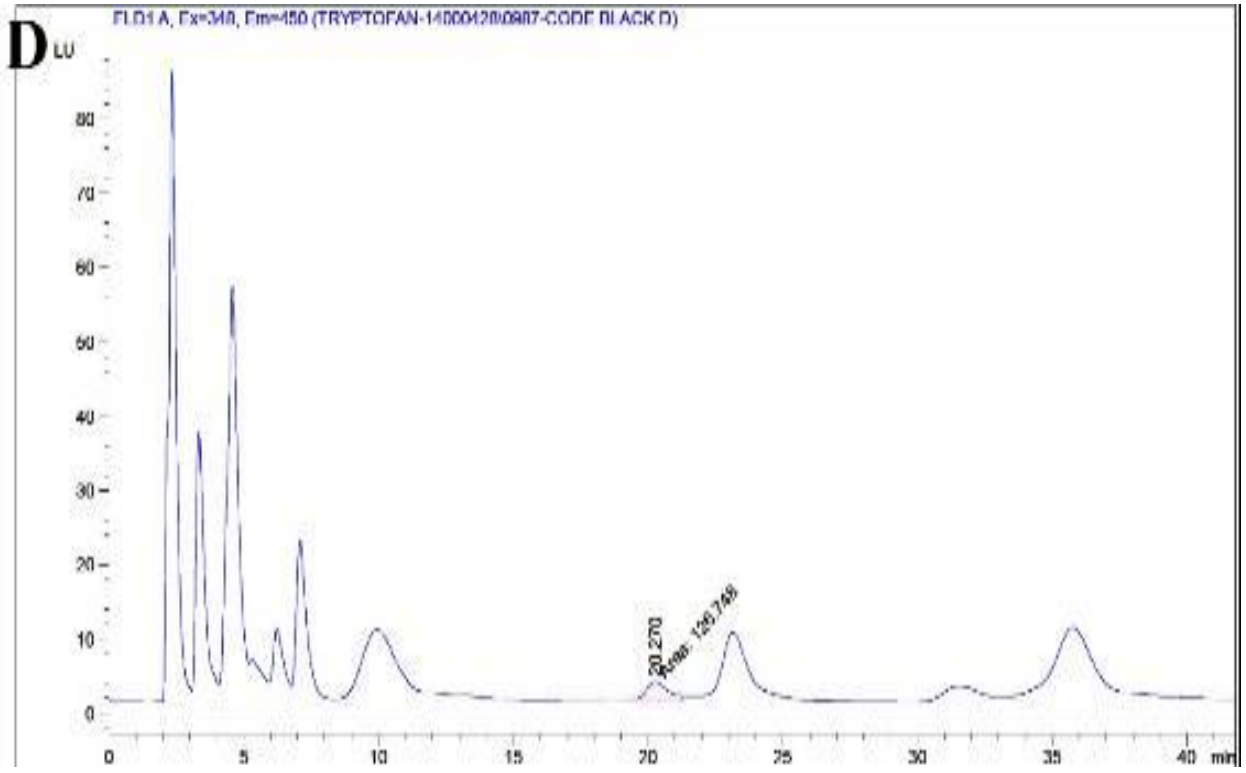

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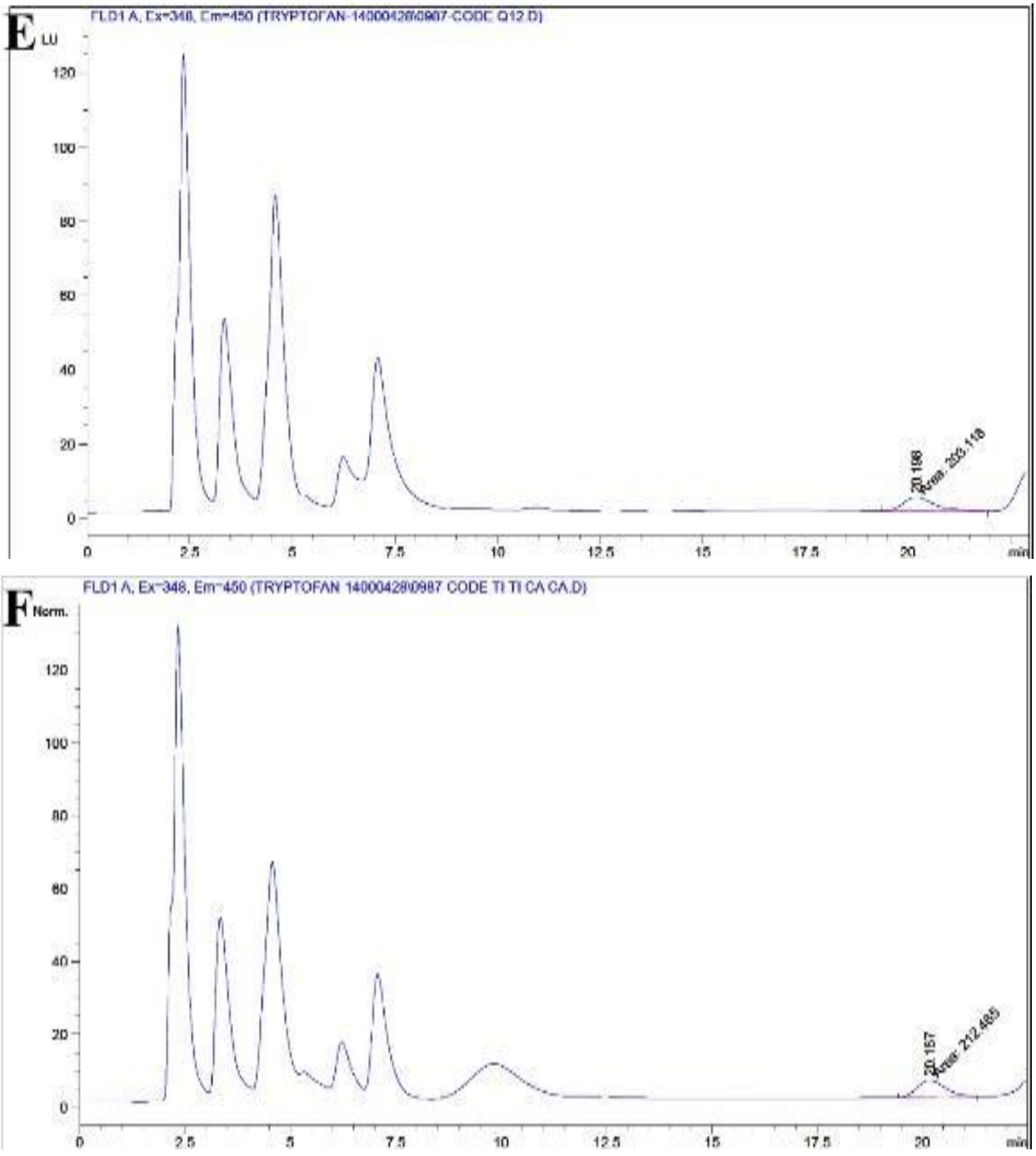


Figure 2. The high-performance liquid chromatograms of three quinoa seed (QS) amino acids; (A) Black-QS; (B) Q12-QS; (C) Titicaca quinoa seed (T-QS); except tryptophan; D, E and f are tryptophan chromatograms of Black-QS, Q12-QS and T-QS genera respectively; Quinoa seeds (QS).

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.

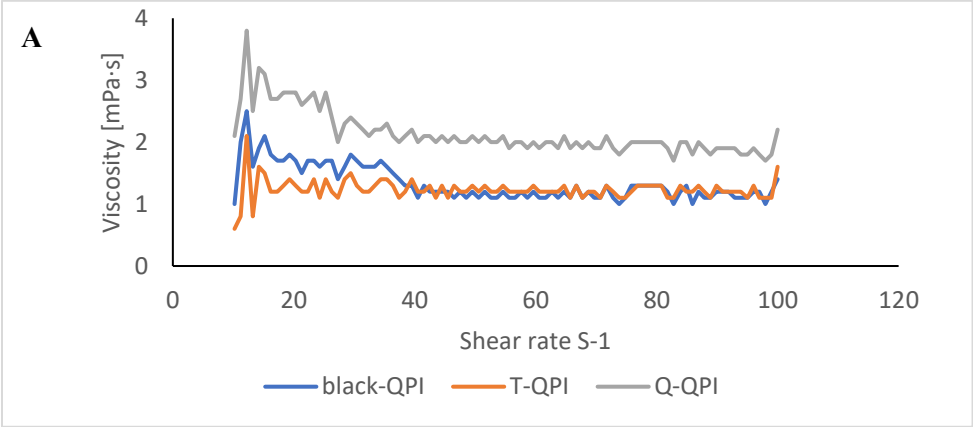
Table 3. Foaming capacity and stability (Mean ± SD, n = 3) of quinoa protein isolated (QPI), (Black, Q12, and Titicaca)

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±5.44b^{DE}	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$^{\underline{b}}$	79.77±3.90$^{\underline{a}}$	76.04±3.89$^{\underline{a}}$	55.91±7.73$^{\underline{a}}$	45.00±6.04$^{\underline{c}}$	40.54±6.12$^{\underline{a}}$
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$^{\underline{a}}$	74.28±6.30$^{\underline{b}}$	51.99±4.84$^{\underline{b}}$	44.77±5.76$^{\underline{b}}$	39.81±5.25$^{\underline{b}}$	35.58±5.99$^{\underline{b}}$
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$^{\underline{a}}$	77.80±3.99$^{\underline{a}}$	59.55±4.62$^{\underline{a}}$	51.18±5.8$^{\underline{a}}$	44.91±5.90$^{\underline{a}}$

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 ($P \leq 0.05$).

Viscosity of QPIs

The oscillatory rheology of QPIs is shown in Fig 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 Fig 3B.



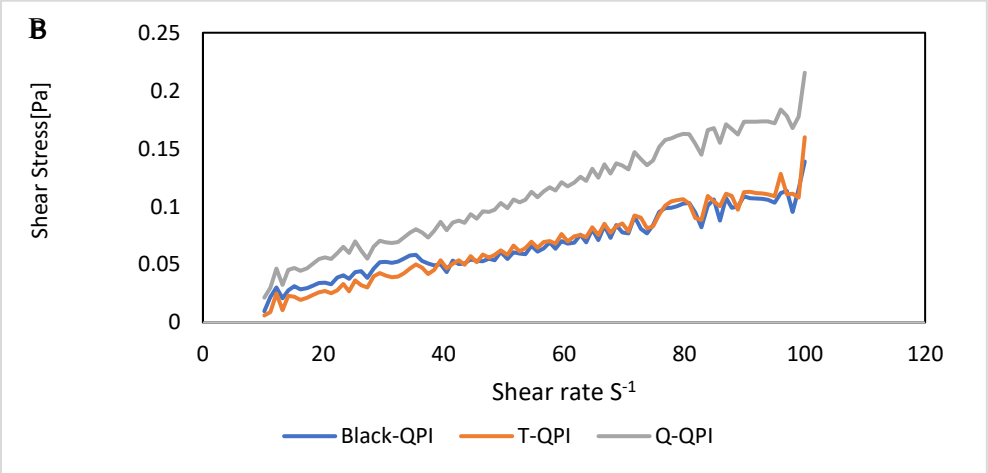


Figure 3. (A) The apparent viscosity; (B) Shear stress versus shear rate curves of QPIs samples (Black-QPI, Q12-QPI, T-QPI); Quinoa protein isolate (QPI); Titicaca (T)

Water and oil absorption of QPI

In the present study, QPIs showed water and oil absorption (1.0±0.06 to 2.02±0.02 ml/g) and (2.0±0.02- 3.02±0.03 ml/g), respectively (Table 4). The water absorption capacity of T-QPI (2.02±0.02 ml/g) was the highest among the others. Water absorption is a characteristic of protein in viscous foods like soups, baked goods, and dough. Therefore, T-QPI may be useful in these food formulations. The oil absorption capacity of Black-QPI (3.02±0.03 ml/g) was the highest among the others.

Table 4. Oil, and water absorption parameters (Mean ± SD, n = 3) of quinoa protein isolated (QPI) of three genera (Black, Q12, and Titicaca)

parameters		Black-QPI	Q12-QPI	T-QPI
Water and Oil absorption	WA (ml/g)	1.9±0.01b	1.0±0.06c	2.02±0.02a
	OA (ml/g)	3.02±0.03a	2.0±0.02c	2.42±0.03b

WA: Water absorption; OA: Oil absorption; Quinoa protein isolate (QPI); Titicaca (T).

DISCUSSION

Proximate analysis of quinoa seeds and QPI

All types of quinoas had adequate amount of protein in this research. There was a correlation between high protein content and potential binding capacity. For instance, an increase in the water absorption of the semolina showed after increasing the protein content of the product (Sissons et al., 2021). The observed protein content of (14.93±0.21 to 16.40±0.22) for quinoa in this study corresponded to that of Gómez et al. (2021) results that reported a protein content range of (15.59-18.73%).

According to the FAO/WHO/UNU standards for protein quality, quinoa protein can provide substantial excesses of several essential amino acids relative to 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 amino acids—methionine and cysteine—compared to most other plant sources. The overall profile of essential amino acids in quinoa surpasses that of 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 (Dini et al., 1992). 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 in this study (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% ± 0.11) (Saavedra & Carmen Valdez-Arana, 2021).

Starch is the primary carbohydrate component in quinoa, accounting for between 52% and 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 between 1.3% and 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 precentage 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, fiber, healthy fats, and carbohydrates, quinoa can contribute meaningfully to balanced diets when consumed alongside a variety of other foods (Elsohaimy 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 which are generally observed in limited amounts in other common grains (Dakhili et al., 2019).

In amino acid measurement, different findings are shown by Gómez (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 in pigeon pea and chickpea (7.45–7.90 %) varieties compared with QPIs (≤0.06-0.3%). While, the values of tryptophan were higher in QPIS (6.55-8.23%) than 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±1.77 to 76.93±3.36% for Black-QPI, 43.09±3.09 to 55.39±0.43% for Q12-QPI, and a constant value of 50.00±0.35% for T-QPI. Among all samples, Black-QPI exhibited the highest average foaming ability (65.26±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±3.86 to 84.50±1.64 % for Black-QPI, 67.92±1.62 to 83.33±5.01% for Q12-QPI, and 78.46±1.66 to 88.68±4.43% for T-QPI, with T-QPI showing the highest average (82.96±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 between 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 (Steffolani et al., 2016). 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. High 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 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 flavor. In similar research, the 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 protein), respectively. Recently, Reséndiz et al. (2019) studied the oil absorption capacity of QPIs and discovered that

QPIs had a 2.66 mL/g value, which this data 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 mouthfeel of food. This indicates that Black-QPI may have stronger flavor retention than 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.

CONCLUSIONS

In conclusion, significant differences in the chemical composition, structure, and rheological properties of quinoa protein isolates (QPIs) from three varieties were identified —Titicaca (T-QPI), Q12 (Q12-QPI), and Black (Black-QPI)—likely due to inherent seed composition differences. Q12-QPI showed the best rheological performance, suitable 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, with future research needed to enhance processing methods that maintain protein quality across genotypes.

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

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چکیده

کینوا یک شبه غله است که اخیرا 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 پروتیین کینوای سیاه و تیتیکاکا به ترتیب (87/30±1/96، 1/161± 87/80٪ وزنی /وزنی) بوده است. نتایج نشان داد در پروتیین کینوای سیاه ظرفیت کف کردن (40/54 درصد)، پایداری کف (65/26 % در 60 دقیقه) و جذب روغن (3/02 میلی‌لیتر بر گرم) به طور معنی‌داری ($p\leq0.05$) 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.