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# Production of Protein Isolate from Quinoa and Studying of Its Some Physiochemical Properties

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**ABSTRACT:** Determining the protein isolate's (PI) chemical makeup, amino acid profile, FTIR, and functional characteristics was the aim of this investigation. Protein, fat, fiber, ash, carbs, and moisture are all present in large amounts in QPI (94.25, 2.43, 0.0, 1.83, 1.56, and 5.92%, respectively).
In this work, we used the techniques of alkaline solubilization and acid precipitation to extract proteins from white quinoa with the goal of understanding how the extraction pH (11) affected the quinoa protein isolate's (QPI) recoverability, purity, and rate of recovery. According to the results, protein purity was 81%, protein extractability was 56.45%, and recovery was 86%. At pH 7 and 11. At PH 7, the maximum solubility for suspension was 67%.

## INTRODUCTION

Due to its excellent nutritional protein quality and quantity, quinoa is considered a plant that can be regarded as a whole meal. As a result, quinoa has become one of the most promising protein sources, suitable for extraction and use as raw material in the food industry to create new food products.
Quinoa (*Chenopodium quinoa willd*) is a remarkable crop with centuries of natural cultivation in South America [Jacobsen, 2003], and it has been grown for over 7,000 years. Its exceptional nutritional value and versatility have driven a sharp increase in its harvested area worldwide, transforming it from a minor crop into a major one [Bazile, 2016].
Protein, as a macronutrient, is vital for maintaining human health and providing structural attributes to food through gelation, emulsification, and foaming. Quinoa contains all nine essential amino acids [Navruz-Varli and Sanlier, 2016].
Quinoa seeds, due to their high protein content and balanced amino acid profile, are widely used in vegan diets. They provide an optimal amino acid balance, rich in lysine and thionic amino acids, which are essential for human life. Quinoa seeds can be boiled to make baby cereal or consumed as a hot breakfast cereal, replacing rice. Pulverized seeds can also be used to make noodles, bread, pasta, and biscuits [Valencia-Chamorro, 2003].
Filho et al. (2017) noted that quinoa seeds are suitable for celiac patients because they are gluten-free and have a low prolamin concentration. Quinoa is among the few plants that provide all essential amino acids for human health and is also rich in thionic and lysine amino acids. Quinoa seeds can be boiled for baby cereals or breakfast, and ground seeds can be used to make bread, pasta, noodles, and biscuits [Valencia-Chamorro, 2003].
Additionally, quinoa is highly valued for its nutritional quality, containing 12–17% protein and a well-balanced amino acid composition [Stikic, 2012; Miranda, 2012; Nowak, 2016; Vega, 2010; Wang, 2020]. Its low prolamin concentration also makes it suitable for individuals with celiac disease [Dakhili, 2013; Burrieza, 2019; Caio, 2019].
The protein content in quinoa can be used to improve the functional and nutritional properties of protein-based foods and beverages. Föste et al. [2015] pointed out that allergies and intolerances have led scientists to replace traditional animal-based proteins, such as milk and eggs, with legumes and other plant-based proteins. However, certain legumes contain gliadins and glutenins, which are associated with celiac disease.
Given quinoa's low gluten content and high protein levels, it serves as an excellent alternative. Various methods, such as precipitation and solubilization, can be used to extract protein from quinoa seeds. In the past decade, pseudo-cereals have gained popularity in healthy diets and diets designed for people with cereal allergies [Gorinstein et al., 2008].

## MATERIALS AND METHOD

We bought the quinoa seeds (*chenopodium quinoa*) from the nearby Baghdad market. Before being kept in polyethylene bags in a dry place at room temperature (about 25°C) for additional analysis, the seeds were cleaned of any extraneous contaminants.

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**Quinoa protein Extraction**

Whole grains were washed with cold (2°C) water until the supernatant was completely foamed-free and then were dried in an air-draft oven at 45°–1°C to remove saponins. Whole seeds were ground with a Miller and sixty mesh screens (Abugoch et al. 2008).

**Defatted quinoa flour Preparation**

Shaking for 1 h at room temperature and then repeating this process four times using 4: hexane (w/v) ratio.The residual hexane was removed overnight in the fume hood to room temperature from the defatted flour.Defatted flour suspended 10% w/w deionized water at pH 11.0 was corrected.

Agitation at 25 °C and 300 rpm for 90 minutes was performed on the suspensions, and then centrifuged for 15 minutes at 9000 x g and 25 °C.Extraction suspensions were prepared by filtering the supernatants through 200 mesh nylon gauze filters.Lowing of pH to 4.5 of the extraction suspensions was used to precipitate the quinoa protein and this was centrifuged at 9000 x g and 25 °C for 15 min. After precipitate was re suspended in deionized water and then centrifuged for 15 minutes at 25° C and 9000× g. The precipitated proteins were then neutralized with 1/6 M NaOH and again suspended in deionized water. Freeze dried QPI was obtained and freeze-dried QPI protein isolates were ground and moistened to greater than 40% moisture to obtain QPI powder.

**Proximate composition**

The quinoa seed flour and its protein isolates were analyzed by the AOAC [2023] to determine the proximate composition (moisture, fat, protein, total carbohydrate, crude fibre and ash).

**Protein solubility**

The solubility of quinoa protein isolate was assessed across a pH spectrum from 1.00 to 10.00. A suspension containing 5% protein isolate was prepared. and stirred at room temperature of 25°±2°C for one hour. at different achieved pH levels (data not shown) to enhance solubilization. The pH was adjusted with 0.1 N HCl and 0.1 N NaOH solutions.

The supernatants were collected and assessed according to the Kjeldahl method to measure the total nitrogen to estimate the total protein content after centrifugation at 6000 g at 20 °C for 30 min at various pH values. The solubility curve for the protein at each Ph value examined was constructed based on the average results obtained [Aluko and Yada (1993)].

**Determination of Protein Extractability and Purity**

Protein levels in quinoa flour and QPI were measured using the Kjeldahl method with a conversion factor of 5.85. The Bradford assay was used to measure protein concentration in QPI suspension supernatants. Extractability, purity, and recovery rate of quinoa proteins were calculated using specific equations:

- **Extractability (%):**

$$\text{Extractability (\%)} = \frac{\text{Extraction suspension volume (mL)} \times \text{Suspension protein content (g/mL)} \times 100}{\text{Flour protein content (\%)} \times \text{Flour weight (g)}}$$

- **Protein Purity (%):**

$$\text{Protein Purity (\%)} = \left( \frac{\text{Isolate protein content (\%)} \times \text{Isolate weight (g)}}{\text{Isolate weight (g)}} \right) \times 100$$

- **Recovery Rate (%):**

$$\text{Recovery Rate (\%)} = \left( \frac{\text{Isolate protein content (\%)} \times \text{Isolate weight (g)}}{\text{Extraction suspension volume (mL)} \times \text{Suspension protein content (g/mL)}} \right) \times 100$$

**Determination of DPPH Radical Scavenging Activities**

The antioxidant activities of quinoa extracts were examined using the DPPH assay, which measures the ability of extracts to scavenge free radicals. Because of the light sensitivity of DPPH, volumetric flasks were covered with aluminum foil to prevent the color degradation from light exposure. Quinoa extracts (150 µL), a control (150 µL water) and a blank (200 µL methanol) were prepared for the assay. In each tube except the blank, 50 µL of 100 µM DPPH solution (100 µM final concentration) was added. An aluminum foil cover was placed over the samples and the samples were incubated at 37 °C for 30.

The handling of samples that were strongly colored remained precarious because they were transparent despite both the presence of aluminum foil wraps and the incubation of the samples in an aluminum foil lidded Erlenmeyer flask. Spectrophotometric absorbance measurement was taken at 517 nm.

- **Inhibition (%):**

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where:

- $A_0$ is the absorbance value of the control.
- $A_1$ is the absorbance value of the extract sample.

**Amino acid analysis**

A high-performance amino acid analyzer (AAA 400, INGOS Ltd., Czech Republic) was employed to conduct the amino acid analysis, adhering to the established protocols by Durrum et al. (1958) and Moore et al. (1958).

The amino acid content was calculated using the following formula:

- **Percentage of Amino Acids (% AA):**

$$\%AA = \left(\frac{\text{Area under the peak (\%)} \times \%\text{protein}}{100}\right)$$

**FTIR analysis**

FTIR analysis was conducted using potassium bromide (KBr) pellets, prepared by pressing 2-mg collagen samples and 200 mg potassium bromide (KBr) under dry conditions. The spectral range used was 400–4000 $cm^{-1}$ [Muyonga, 2004], analyzed with a SHIMADZU (Japan) model apparatus.

**Determination of Protein Solubility**

Shengnan Liu 2023 centrifuged the QPI suspension at 1% w/v at 10,000 rpm and 25 °C for 15 min, with a pH value of 7.0. The protein content in this study was assayed by the Kjeldahl method with a conversion factor of 5.85. In the case of QPI supernatants, the content was estimated by the Bradford assay.

Solubility was determined by applying the following equation.

- **Solubility (%):**

$$\text{Solubility (\%)} = \frac{\text{Supernatant protein content (g/mL)} \times \text{Supernatant volume (mL)} \times 100}{\text{QPI protein content (\%)} \times \text{QPI weight (g)}}$$

**RESULT AND DISCUSSIONS**

**Chemical composition**

Data in Table (1) shows the proximate composition of whole quinoa seed flours and quinoa protein isolates. Quinoa seed flour contains remarkable amounts of protein higher than most commonly used cereals. It also contains significant amounts of protein, fats, fiber, carbohydrates, Ash and moisture 15.75, 6.0, 4.86, 69.95, and 10.32%, respectively. Also provides the chemical composition of the protein isolates because here the isolated protein content was found to be 94.24%, this is consistent with the Gaikwad et al. [2021] who showed that the nutritional content of the quinoa seed is very good according to the carbohydrate (61.12±0.31%), protein (15.24±0.25%) and fat (6.1±0.58%).

**Table (1): Chemical composition of quinoa seed flour and protein isolates (gm/100gm sample on dry weight basis).**

| Chemical composition | Flour protein | Protein isolate |
|---|---|---|
| Total protein | 15.75 | 94.25 |
| Fat | 6.0 | 2.43 |
| Total fiber | 4.86 | 0.0 |
| Total Carbohydrate | 69.95 | 1.83 |
| Ash | 3.84 | 1.56 |
| Moisture | 10.32 | 5.92 |

**Antioxidant assay DPPH**

**At 73.54%,** the quinoa exhibited the highest level of radical inhibition activity. The outcome supported the idea that ground quinoa provides better reproducibility of results than seeds by showing that grinding the quinoa improved its consistency. The antioxidant activity of the white quinoa (Peru and Bolivia) extracts is demonstrated by the quinoa's dependent suppression of DPPH.

**Protein extra ability, purity, recovery**

Room temperature defatted (less than 1% fat) quinoa flour was used to extract QPI. Protein extractability at pH 11, indicated by the data in Table 2, was 56.45%, higher than the protein extractability of the sweet quinoa after it soaked for an extended period of time. Recovery of protein from quinoa protein extraction suspensions is accomplished at 86% when the alkali solubilization and acid precipitation method is used.

On a positive note, the purification of these proteins is within range, at 81%, with similar values from many studies on purity of Quinoa Protein Isolate (QPI) proteins obtained by other quinoa varieties extraction procedures. In this area, key references are Van de Vondel (2020) and Ruiz (2016).

According to Wang (2004) and Florence (1980), highly alkaline pH can deprotonate amine groups, ionize carboxyl groups, and cleave disulfide bonds, which helps more protein components disperse or dissolve into extraction suspensions, improving quinoa protein extractability and recovery.

Furthermore, a high alkaline pH acts synergistically by releasing more non-protein impurities, such as fiber and starch, into the extraction process. These impurities, which might have remained separate in lower protein pulp, allow pure protein to move into a highly clear supernatant [Mäkinen, 2016].

Compared to this, QPI yielded significantly low comparative extractability amongst these three: QPI having an equal range of purity over SPI and Pea PI under a similar technique were extracted [Wang, 2004, and Gao; 2020]. This would therefore imply that the structural characteristics of plant proteins were a major issue themselves.

**Table 2: Protein Extractability and Purity of Quinoa Protein Isolate (QPI) and Recovery Rate from Extraction Suspensions at pH 11**

| Treatment | Quinoa Protein isolate (QPI) |
|---|---|
| Extract ability | 56.45 % |
| Purity | 81 |
| Recovery | 86% |

**Amino acids profile**

Table (3) provides the amino acid profile quinoa protein isolate. A protein's amino acid makeup primarily dictates how nutrient-dense it is. Figure (1) displayed the limiting amino acids of the protein isolate and quinoa. According to this study, phenylalanine is the most abundant amino acid in the isolated protein, followed by alanine in second place and leucine in third.

**Table 3: Composition of Amino Acids in Quinoa Protein Isolates**

Result chromatography Table (Uncal - F:\ Quinoa )

| No | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Amount ( µg/gm) | Calculation | Peak type | Compound Name |
|---|---|---|---|---|---|---|---|
| 1 | 2.02 | 7156.25 | 623.25 | 15.90 | Calibration carve | Order | Aspartic acid |
| 2 | 2.81 | 3251.23 | 211.50 | 12.65 | Calibration carve | Order | Asparagine |
| 3 | 3.76 | 5365.02 | 505.99 | 20.14 | Calibration carve | Order | Lysine |
| 4 | 4.09 | 7654.15 | 622.65 | 18.98 | Calibration carve | Order | Serine |
| 5 | 5.08 | 8362.05 | 318.98 | 13.65 | Calibration carve | Order | Threonine |
| 6 | 5.87 | 4632.25 | 601.66 | 14.52 | Calibration carve | Order | Isoleucine |
| 7 | 6.22 | 8962.35 | 325.90 | 21.55 | Calibration carve | Order | Alanine |
| 8 | 6.88 | 4856.08 | 505.90 | 15.90 | Calibration carve | Order | Valine |
| 9 | 7.99 | 6652.11 | 211.55 | 19.80 | Calibration carve | Order | Tyrosine |
| 10 | 8.56 | 7961.05 | 504.91 | 15.65 | Calibration carve | Order | Arginine |
| 11 | 9.31 | 5521.44 | 525.80 | 19.80 | Calibration carve | Order | Cysteine |
| 12 | 10.22 | 5623.2 | 331.55 | 16.56 | Calibration carve | Order | Methionine |
| 13 | 11.48 | 2785.11 | 524.55 | 17.84 | Calibration carve | Order | Proline |
| 14 | 11.94 | 5632.50 | 220.80 | 19.00 | Calibration carve | Order | Histidine |
| 15 | 12.89 | 7451.25 | 525.65 | 21.50 | Calibration carve | Order | Lucien |
| 16 | 13.12 | 7365.98 | 328.98 | 23.65 | Calibration carve | Order | Phenylalanine |
| 17 | 13.92 | 5521.44 | 592.65 | 17.78 | Calibration carve | Order | Glycine |
| | Total | | | | | | |

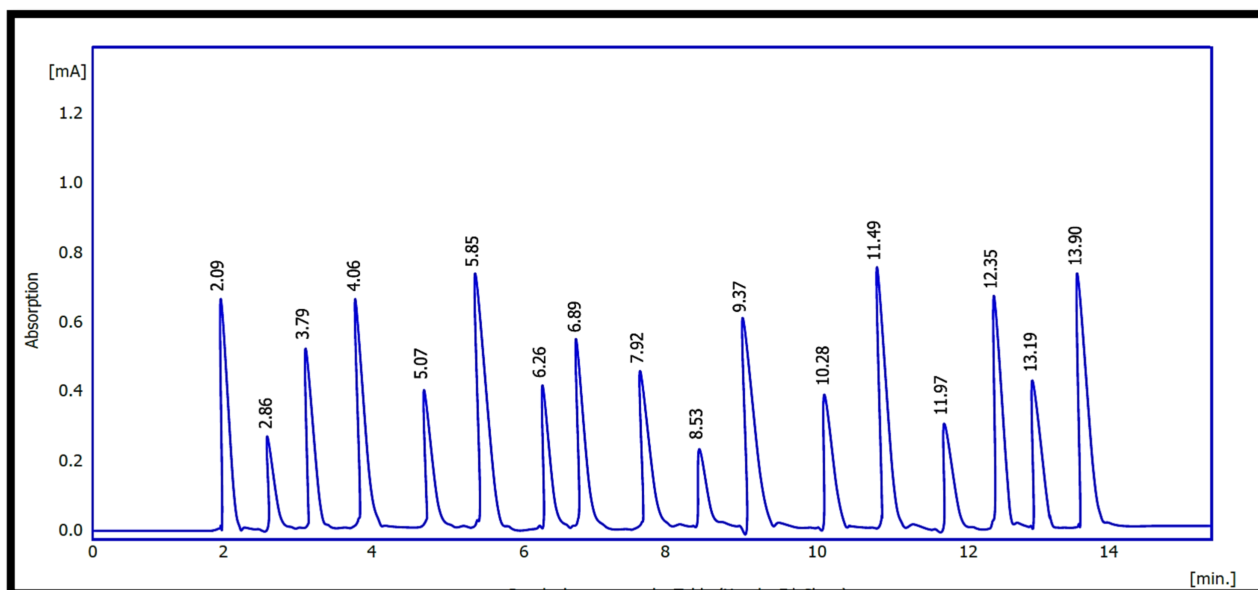


**Figure (1): Amino acids composition of Quinoa protein isolates (QPI).**

In this work, the FT-IR analytical method was used to investigate structural make-up in quinoa protein isolate. Indeed, the main spectral regions of interest that may provide information on flour varieties are changes in the intensity of bands related to lipids at around 3010-2800 cm−1 and 1770-1710 cm−1 [Muik et al., 2007] and those related to proteins at around 1580-1700 cm−1 and 1580-1400 cm−1 [Shotts et al., 2018].

Figure 2 displays spectral intensities at 1,649.02 cm−1 (Amide I) and 1,558.38 cm−1 (Amide II), indicating protein concentration in coproducts. The intensity of Amide I was higher than II.

It should be underlined that the band at 1,558.38 cm−1 was less responsive to protein content compared with the band at 1,649.02 cm−1. However, the latter was more efficient in monitoring the quinoa protein extraction process.

The bands at 1,077 and 1,150 cm−1 were assigned to the C-O-C vibrations functional group from glycosidic bands linked to amylaceous content. Figure 2 also presents the range from 2,800 to 3,000 cm−1, characteristics of lipids [García-Salcedo et al., 2018]. According to Rolandelli et al. [2021], intense bands corresponding to symmetric and asymmetric stretching at 2927 and 2856 cm−1 of CH2 aliphatic groups, respectively, and at 1745.46 cm−1 due to ester carbonyl group stretching, could be related to lipids. There are lipid peaks at about wavenumber 2856.38 cm−1 (symmetric stretching vibration) and 2927.74 cm−1 (asymmetric vibrations), resulting from CH2 acyl groups. The strength of each spectrum altered due to variations in the lipid acyl chains' structural order, enhancing their dynamics [Bet et al., 2018].

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Roasting led to enhanced absorptions at 2,922 cm−1 (asymmetric CH2 stretching vibration) and 2,854 cm−1 (symmetric CH2 stretching vibration) owing to oxidation [Abbas Ali et al., 2017]. In this work we found in FT-MIR spectra changes in the lipid contents for maceration processes, and in agreement with such changes are the changes in the lipid contents after maceration processes. On the other hand, several authors have reported that such modifications in this area of the spectrum of flours can be explained by the amylose lipid complexes formation during the extrusion process. After maceration, the coproducts' amide I and II bands diminish in intensity at periods greater than 24 h. This is because leaching water protein content has increased.
Since co products were substantial variances in protein, starch, and fat, chemical analysis was needed to determine how extraction procedures affected this fraction.

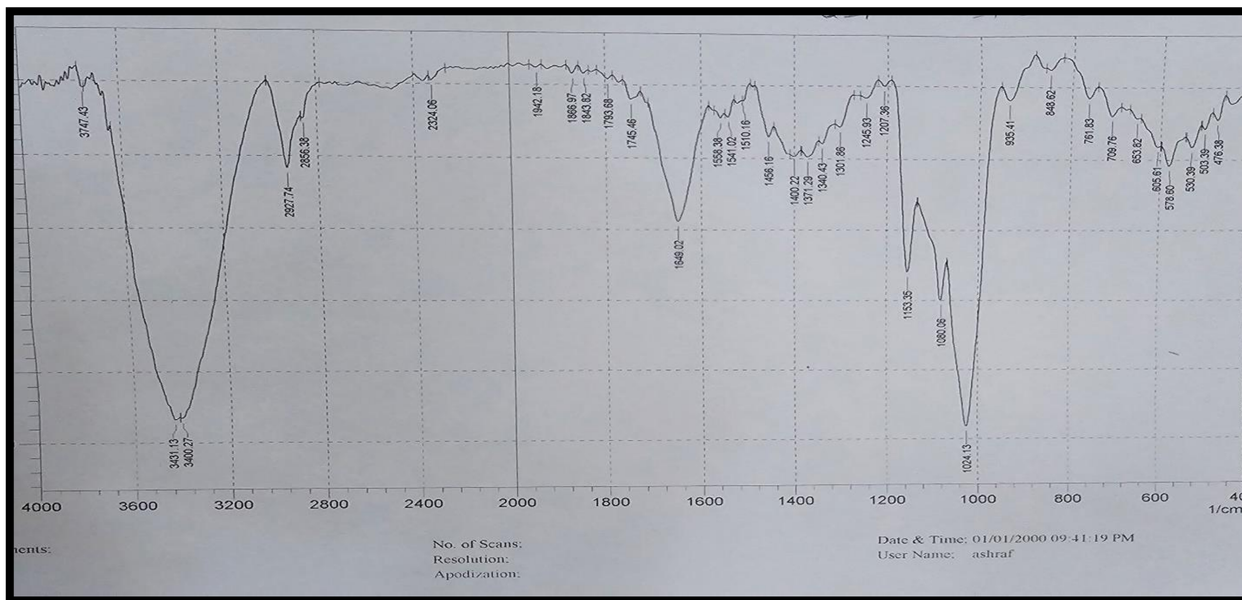


**Figure (2): FTIR of quinoa protein isolate (QPI).**

**Solubility of Quinoa Protein Isolates at Various pH Levels**
QPI solubility at pH 7 was measured and presented in Figure 3, which is highly dependent on its structural properties. From the result, it can be seen that QPI had a remarkable drop from 67% to 45% in its solubility while increasing pH values from 7 to 11. These QPI solubility ranges of 35–70% agreed with those in several previous reports [Ruiz, 2016; Abugoch, 2008; Van, 2021]. QPI extracted at pH 7 had the highest solubility probably because protein extraction was at a similar pH for the determination of its solubility, which may be one crucial factor forming the basis for the reduced solubility of proteins.

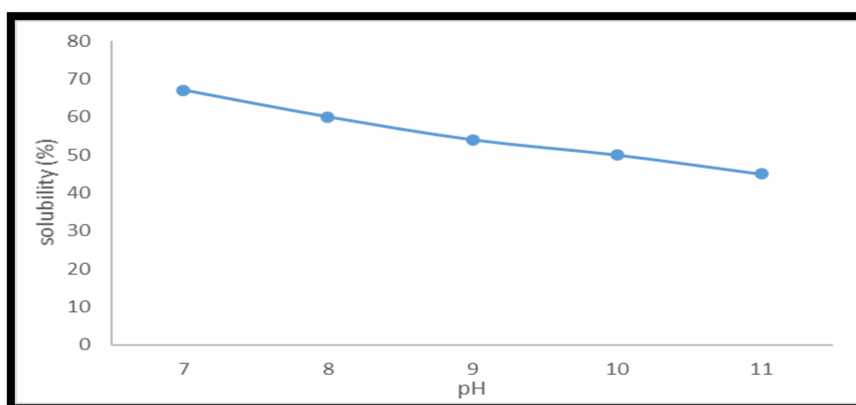


**Figure 3: Solubility of Quinoa Protein Isolate (QPI) Extracted at pH Ranges 7 to 11**

**CONCLUSION**
The FAO has highlighted quinoa as an outstanding source of high-quality protein and dietary fiber. Moreover, quinoa's proximate analysis reveals that it contains essential nutritional components, including proteins and essential amino acids, fibers, fats, and carbohydrates.

If you want to sum it up, quinoa if eaten with moderation with other type of foods could form part of a balance nutrition diet, proteins in the seed of quinoa seeds below those found in legume seed but higher than that found in other cereal grain such as rice.

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