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CHARACTERIZATION, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF QUINOA FLOUR AND ITS PROTEIN ISOLATE

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ABSTRACT: The objective of this study was the determine the chemical composition, amino acids profile, limiting amino acids, sodium dodecyl sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), In-vitro digestibility, and functional properties of quinoa flour (QF) and protein isolates (PI). QF contains significant amounts of protein, fat, fiber, ash, and carbohydrates 14.25, 7.00, 5.14, 3.16, and 70.45%, respectively. Purity of PI was 94.12%, and impurities were about 6%. The first limiting amino acid in QF is cysteine, the second is methionine, and the third is proline. In contrast, in PI the first amino acid is cysteine, the second is histidine, and the third is methionine. The efficiency ratio of quinoa flour proteins (QFP) is higher than that of the PI. The molecular weight (MW) of QFP and PI have similar MW (250,130, 100, 70, 55, 35, and 25 K Da). QFP showed higher digestibility than PI (84.13%, and 79.51%, respectively). QF had a higher significant ($P \leq 0.05$) water absorption capacity than PI. The fat absorption capacity was 1.38 gm oil /gm of flour for defatted quinoa flour and 1.98 gm oil /gram of protein isolates**.** We foundnd that quinoa protein has a high foam stability within 60 min.

Key words: Quality of quinoa protein, Amino Acid, In-vitro digestibility, Anti nutrition factors, Protein classification**,** functional properties.

INTRODUCTION

Formerly here was a misconception that animal proteins were necessary for human growth. For the essential nutrition of the human body, they had a higher amino acid score, higher digestibility, and morewater solubility (Balandrán-Quintana *et al.*, 2019).

Quinoa is one of the promising plants from which protein may be extracted and used in the food industry for many new food items due to its high nutritional protein quality and quantity, which might be considered to be a complete food (Wu, 2015). All nine essential amino acids are present in quinoa (Navruz-Varli and Sanlier, 2016). Due to their high protein and well-balanced amino acid content, quinoa seeds are frequently utilized in the vegan diets, according to Thakur and Nimbalkar (2020). Quinoa seeds are excellent for celiac patients because it does not contain gluten and have a low concentration of prolamins, according to Filho *et al.* (2017) quinoa is one of the few plants that contains all the amino acids required for human life, has a perfect amino acid balance, and is rich in thionic and lysine amino acids. Quinoa seed can be used instead of rice, as a hot breakfast cereal, or for manufacturing baby cereal by boiling it in water. The seeds can be ground into flour and used to make pasta, bread, noodles, and biscuits (Valencia-Chamorro, 2003).

According to Föste *et al.* (2015), scientists have substituted legumes and other plant-based proteins, with typical animal-based proteins like milk and eggs because of allergies and intolerances. However, the presence of gliadins and glutenins in several legumes is linked to the onset of celiac disease. Quinoa seed can be used a good substitute because it has a high protein content and less gluten. Quinoa seed protein can be extracted using several techniques, such as precipitation and solubilization. The usage of pseudo-cereals has expanded over the past ten years, both in healthy diets and special diets for those allergic to cereals (Gorinstein *et al.*, 2008). As a result, quinoa seed is getting much attention

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as an alternative crop worldwide (Peruto *et al.*, 2001).

This study was focused on quinoa seed flour and protein isolates. This research amied to study chemical composition of seeds, nutritional value and the functional characteristics of protein isolates and quinoa flour.

MATERIALS AND METHODS

Materials

Quinoa seeds (chenopodium quinoa) were obtained from the National Center for Agriculture Research 2021. The seeds were purified and foreign materials before being stored in polyethylene bags in a dry location at room temperature (about 25^{o}C) for further examination.

Methods

Samples preparation

Whole Quinoa Seed

To remove saponins, whole seeds were rinsed in cold (2°C) water until there was no more foam, and they were then dried in an air-draft oven at 45°–1°C until dry. Using a Miller (Proctor Silex model E160, UPC) and a sixty-mesh screen, the whole seeds were ground (Abugoch *et al.*, 2008).

Defatted quinoa flour Preparation

Ground quinoa seed flour was defatted by soaking four times with n-hexane (60-80°C) at room temperature for 48 hrs. The solvent was changed every 12 hrs. The defatted flour was dried until all traces of hexane were removed at room temperature (25°C). The defatted quinoa seed flour passed through a 25 mm (British standard screen) sieve, the material was milled once more. The fine flour kept in plastic (polyethylene) pages in the deep freezer (-18 °C).

Preparation of quinoa protein isolates

Quinoa protein isolate was prepared according to Alsohaimy *et al.* (2007).

Proximate composition

The proximate composition (moisture, fat, protein, total carbohydrate, crude fiber, ash, and mineral contents) of quinoa seed flour and its protein isolates were determined according to AOAC 2023.

Amino acid analysis

According to Durrum *et al.* (1958) and Moore *et al.* (1958), the samples were subjected to an amino acid analysis using a performance amino acid analyzer (AAA 400, INGOS Ltd., Czech Republic). And calculated according to the following equation:

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

Protein Efficiency Ratio (PER) a = 0.456 + 0.454 (Leucine) - 0.047 (pro) and Protein Efficiency Ratio (PER) b = 0.498 + 0.454 (Leucine) - 0.105 (Tyrosine) were calculated (Alsmeyer *et al.* 1974).

SDS–PAGE of quinoa protein

According to Laemmli (1970), the protein composition of quinoa protein was determined using Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) with 5% stacking gel and 12% separating gel.

In vitro digestibility

Using the multi-enzyme approach of Bodwell *et al.* (1980) and Carbonaro *et al.* (1997). The in-vitro protein digestibility was calculated using the equation below:

$$Y = 234{,}84 - 22{,}56\ X$$

Where

- Y is the in-vitro digestibility of protein (%).
- X is the pH of the suspension after 20 min digestion.

Protein classification

Using a modified version of the Osborne classification process as reported by Lund and Sandstorn (1943), protein classes of quinoa protein isolates were divided according to their solubility. The Kjeldahl method was used to determine the protein content of the collected

supernatants total nitrogen content as well as the residue left over after successive extractions. Each protein fraction's content was calculated as a percentage of the meal's overall nitrogen content, which is the entire nitrogen content of all its components, including residue.

Determination of anti-nutrients

Determination of saponins

The standard procedure of Obadoni and Ochuko (2002) and Rodriguez (2017) was modified slightly. By using the following equation, the amount of saponins, given in percent, was estimated.

$$\% \text{ saponin} = \frac{\text{weight of residue}}{\text{weight of sample}} \text{ x } 100$$

Determination of phytic acid

The measurements were done using a modified version of the Wheeler and Ferrel (1971) and Kayode *et al.* (2013) methods.

$$Phytic\ acid(\%) = \frac{Tv \times 1.19 \times 3.55 \times N \times DF}{W} \times 100$$

Determination of Tannin Content

The modified vanillin-HCl method of Price *et al.* (1978) was used to quantitatively measure tannin as a gallic acid equivalent.

Functional properties

Oil and water absorption capacity

The method of Sathe and Salunkhe (1981) was used to determine the amounts of oil and water absorbed the quinoa protein isolate and quinoa flour.

Foaming capacity and stability

The foaming characteristics of quinoa seed flour and protein isolate were assessed using the techniques outlined by Tsutsui (1988) and Shahidi *et al.* (1995). The formula below was used to compute foam capacity:

$$\text{Foam expansion (\%)} = \frac{(A-B)}{B} x100$$

Where

- A= volume after whipping (ml) at different times and
- B= volume before whipping

Emulsion capacity and stability

Pearce and Kinsella (1978) methed was used to determine the stability and emulsion capacity. The emulsion activity index (EAI) and the emulsion stability index (ESI) were calculated according to the following equation:

$$EAI\ (m^2/g) = (2x2.303xA_o) - (.25 \text{ x protein concentration})$$

Where

- Ao = absorbance measured immediately after emulsion formation at 500 nm.

$$\text{ESI (min)} = \frac{\text{Ao } x\Delta t}{\Delta A}$$

Where

- ΔA =Ao - A10 and Δt = 10 min.

Statistical analysis

All data will be shown as mean SD (standard deviation). Costat version 6.311 (Copyright 1998-2005, Cohort Software) (SAS, 2000) was used for the statistical analysis. Except for the emulsion characteristics and foaming ability of quinoa flour and protein isolates, every analysis is reported as the variance (one-way ANOVA) for all results. At 5% probability (P 0.05), differences between treatments were considered significant.

RESULTS AND DISCUSSION

Proximate 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, and carbohydrates 14.25, 7, 5.14, 3.16, and 70.45%, respectively. Also shows the chemical composition of the protein isolates, where, the isolated protein content was found to be 94.12%, and the ingredients and impurities are about 6%, this results consistent with Gaikwad *et al.* (2021) who found that the quinoa seeds have a good nutritional profile with 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 Constituents	Quinoa seed Flour	Protein Isolate	LSD
Total Protein (N x 6.25)	$14.25^{b} \pm 0.92$	$94.12^{a} \pm 0.82$	1.978
Crude Lipids	$7.0^{a} \pm 0.42$	$2.33^{b} \pm 0.09$	0.688
Crude Fiber	$5.14^{a} \pm 0.04$	$0.0^{b} \pm 0.0$	0.065
Total ash	$3.16^{a} \pm 0.41$	$1.66^{b} \pm 0.04$	0.253
Total Carbohydrates	$70.45^{a} \pm 0.29$	$1.89^{b} \pm 0.25$	0.619
Moisture	$11.31^{a} \pm 0.71$	$6.03^{b} \pm 25$	1.218

Means in the same row with different letters are significantly different ($p \leq 0.05$).
*Means ± standard deviation of means of three determinations.
LSD = Least Significant Different

Amino acids profile

Table (2) represents the amino acid profile of quinoa seed flour and quinoa protein isolate. A protein's amino acid makeup mostly determines how nutrient-dense it is. Quinoa seeds had an amino acid profile similar to milk casein. Our findings were consistent with those reported by Bhargava *et al.* (2003), who discovered that quinoa protein contains larger amounts of lysine (5.10–6.4%) and methionine (0.4–1%).

limiting amino acids of quinoa and protein isolates

The limiting amino acids of quinoa and protein isolates were shown in Table (3). This study showed that the first limiting amino acid in quinoa flour is cysteine and the second amino acid is methionine, and the third amino acid is proline, while in the isolated protein, the first amino acid is cysteine, and the second amino acid is histidine, and the third is methionine. The efficiency ratio of quinoa protein flour (15.7) is higher than the isolated protein (14.9), this study is similar to Ranhotra *et al.* (1993) who reported that the PER of cooked quinoa was 30% greater than that of uncooked quinoa. Also they concluded that the quality of protein in quinoa is equals to that of casein.

Sodium- dodecyl sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) pattern of defatted quinoa flour and protein isolates

To determine the polypeptide chains of the major proteins in defatted quinoa flour and protein isolate, the sample was subjected to electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS). The molecular weights of these proteins are shown in Fig. (1). This study showed that the molecular weights of flour and isolated protein have similar molecular weights (MW) 250,130, 100, 70, 55, 35, and 25 K Da, this study is similar to that reported by (Shen *et al*., 2021) who showed that QPI mainly consists of globular and albumin proteins and shows a complex protein band profile. Similar protein bands were observed in recent electrophoresis studies on the QPI obtained by alkaline extraction methods.

In-vitro proteins digestibility in quinoa flour and protein isolates

Quinoa flour and protein isolates in-vitro protein digestibility is shown in Fig.2. One of the most crucial factors affecting the quality and usage of protein in the human body is in-vitro protein digestion, which was significantly

affected by quinoa flour and protein isolate (P> 0.05). In this study, quinoa flour proved high digestibility, which determines its quality, but there was a slight difference between flour protein digestibility, and isolated protein, Quinoa flour showed higher digestibility than isolated proteins 84.13%, 79.51% respectively, as shown in Fig.2. The findings of this study are in line with those of other studies by Zia-Ur-Rehman and Shah (2001) and Repo-Carrasco-Valencia and Serna (2011), according to which the digestibility of proteins is a crucial factor in determining how nutrient-dense they are. In-vitro digestibility of quinoa protein isolate was found to be 78.37±1.08%.

Table (2): Amino acids composition of Quinoa seeds flour and protein isolates.

	Amino Acid	Results (Amounts mg/gram Protein)		FAW/WHO (2007) mg/gm
		Deffated quinoa flour	Protein Isolates	
Essential Amino acid	Histidine	36.88	16.10	15
	Leucine	42.94	46.39	59
	Lysine	96.10	62.24	45
	Isoleucine	33.40	22.36	30.00
	Threonine	33.81	31.32	23
	Valine	42.09	34.75	39
	Methionine	18.98	2.25	———
	Phenylalanine	41.10	41.06	———
	Tyrosine	41.00	63.33	———
Non Essential Amino acid	Alanine	39.59	36.79	———
	Aspartic acid	199.36	217.62	———
	Arginine	86.91	84.94	———
	Glycine	53.80	39.67	———
	Glutamic acid	132.47	148.59	———
	Cysteine	2.25	3.23	———
	Proline	27.81	28.13	———
	Serine	53.80	56.75	———

Table (3): limiting amino acids of quinoa and protein isolates .

Materials	Limiting amino acid			PER
	First	Second	Third	
Defatted quinoa flour	Cysteine	Methionine	Proline	15.687
Protein isolate	Cysteine	Histidine	Methionine	14.9

PER = protein efficiency ratio

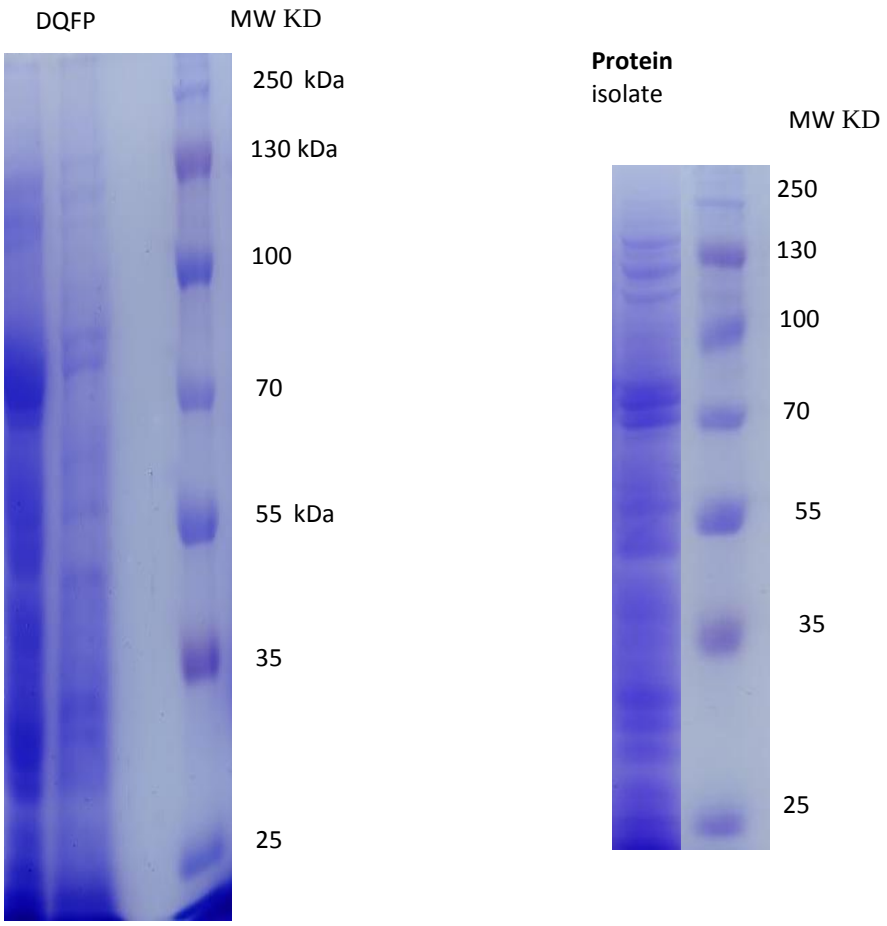

Fig. (1): SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) pattern of defatted quinoa flour and its protein isolates

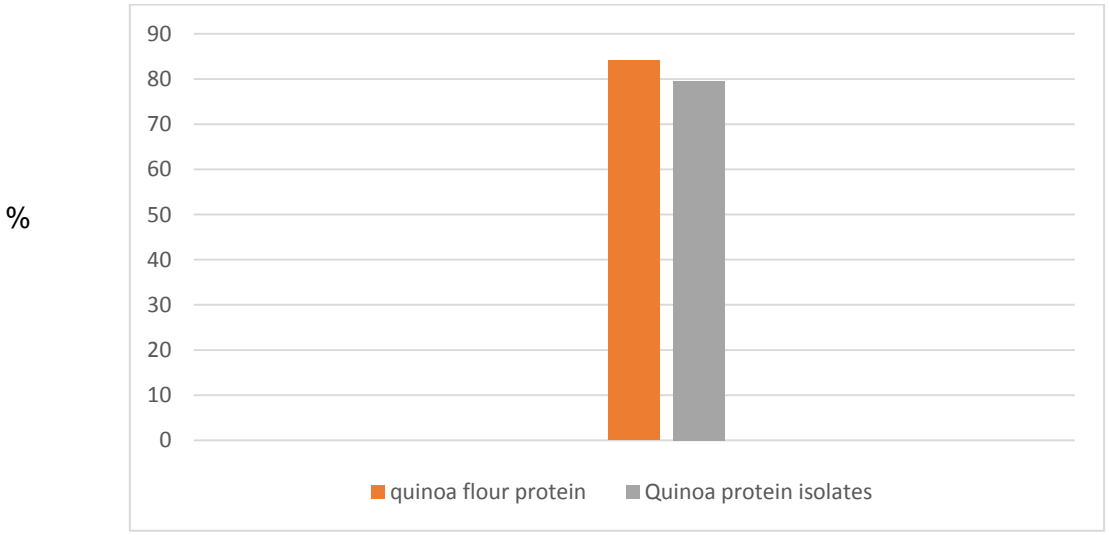

Fig. (2): In-vitro proteins digestibility in quinoa flour and protein isolates (%)

Effect of different solvents on the protein solubility index

Data are provided in (Fig. 3) for the determination of the protein solubility index of quinoa protein isolates in distilled water, 0.1 M sodium chloride, 70% ethanol, and 0.1 M sodium hydroxide. According to this investigation, the most protein was soluble in 0.1M sodium hydroxide, followed by distilled water, 0.1M sodium chloride, and finally 70% ethanol. The quinoa protein had a low water solubility, with a maximum solubility of only about 21.1%, according to a similar study of Tavano *et al.* (2022).

The solubility studies of quinoa protein isolates proved that the major protein is globulin (30.99%) followed by albumins (22.14%) as storage protein. However prolamins was very low (2.26%) only.

Anti-Nutrition factors in defatted quinoa flour and protein isolates

One of the main limiting factors that affect the nutritional and food characteristics of legumes and some grains like quinoa seeds is the presence of anti-nutrition compounds. Table (4) displays the anti-nutrition characteristics of protein isolate and defatted quinoa flour. Non-significant ($P < 0.05$) differences between defatted quinoa flour and protein isolates in this regard. This study showed that saponins and phytic acid are found in quinoa flour in a greater amount than in isolated protein, but the tannin is found in a greater amount in isolated protein because the tannin in nature is found in a complex form with protein, Flour is high in content of both saponins and phytic acid while protein is high in tannin and low saponins and phytic acid, this means that water washing removed a large portion of the saponins due to its water solubility during the preparation of PI. This study was in agreement with earlier research that measured the levels of saponins in three different kinds of Salcedoe Regalona cultivated in Chile, Peru, and Spain. Saponin content ranged from 8 to 13 g kg^{-1}, but there were no appreciable differences. As a result, it was hypothesized that this attribute is more strongly influenced by the genotype than by the environment Reguera *et al.* (2018).

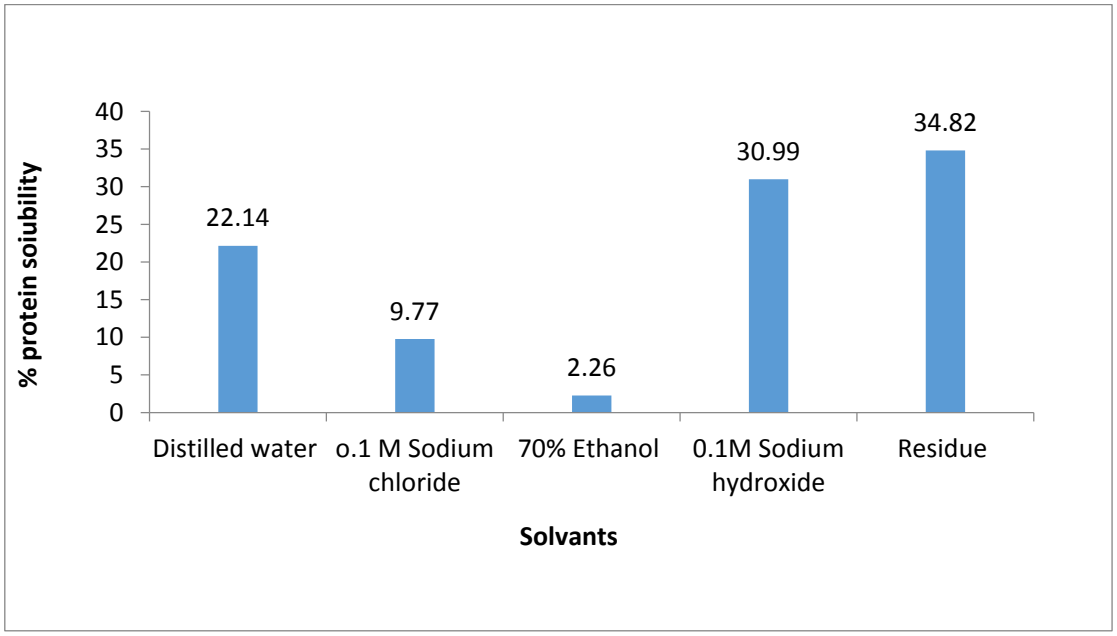

Fig. 3. Protein solubility index of protein Isolate from quinoa in different solvents.

Table (4) Anti-nutritional factors of quinoa flour and its protein isolates

Anti-nutritional compounds	Flour	protein isolate	LSD
Saponins %	$3.96^{a} \pm 0.152$	$1.90^{b} \pm 0.1$	0.292
Tanine %	$0.49^{b} \pm 0.02$	$0.87^{a} \pm 0.03$	0.057
Phytic Acid %	$1.23^{a} \pm 0.152$	$0.018^{b} \pm 0$	0.245

Means in the same raw with different letters are significantly different ($p \leq 0.05$).
Means ± standard deviation of means for three determinations.
LSD = Least significant difference

Physicochemical properties and techno-functional properties of DQF and QPI

Water absorption capacity

Absorption of water for defatted quinoa flour and isolated protein is found in Fig. 4. DQF had a significant ($P \leq 0.05$) higher water absorption capacity than QPI. The values for defatted quinoa flour and isolated protein were 1.81 and 1.46 gm H_2o /gm flour: protein, respectively. According to (Dakhili *et al.* 2019) QPs absorbed water at a rate of 3.94 ±0.06 ml/g, which is higher than pearl millet and wheat but lower than soy protein.

Oil absorption capacity

Significant ($p < 0.05$) differences were observed between defatted quinoa flour and quinoa protein isolates in their absorption capacities in Fig. 4. The fat absorption capacity was 1.38 gm oil /gm flour for defatted quinoa flour and 1.98 gm oil /gram protein isolate. The oil absorption capacity of QPs was reported by Ashraf *et al.* (2012) to be 1.88 ±0.02 ml/g, which is somewhat higher than wheat but lower than pearl millet and soy protein. These results are comparable to those reported by those authors. The same pattern was shown with oil absorption, as quinoa protein absorbed 1.88 ± 0.02 ml/g, while soy protein absorbed 2.10 ±0.10 ml/g and wheat protein absorbed 1.58 ±0.03 ml/g.

Emulsifying activity and stability

According to Elsohaimy *et al.* (2015), emulsion characteristics are one of the crucial functional traits of proteins that influence how food products behave. The emulsion properties of defatted quinoa flour and protein isolate are found in Table 5. In this study, the isolated quinoa protein proves higher emulsification activity and stability in low concentrations of protein as well as defatted quinoa flour protein which, was in the same behavior. But the isolated protein showed much higher emulsification activity and stability than quinoa flour protein as shown in Table 5.

Foam properties capacity and stability

Foaming capacity

The foam formation of isolated protein and quinoa flour protein is found in (Table 6), and it was observed from this study by increasing protein concentration, the foaming capacity increased, and the maximum foam was found at concentration of 3%, the foaming capacity for quinoa flour and protein isolates at concentration 3% was 35.1, 78.25% respectively. But the isolated protein has shown a much higher foam than the quinoa flour protein, as shown in the table. This study is comparable to (Lomakina and Mikova, 2006), which reported that the foaming capacity (FC) of quinoa protein isolate varied from 58.37±2.14% at 0.1% protein concentration to 78.62 ±2.54% at 3% protein concentration, with an average of 69.28%. The foaming capacity increased considerably as the protein concentration increased (P 0.05). Foaming stability (FS) ranged from 83.55 ±5.95 at 0 min to 54.54 ±15.31% at 60 min (P 0.05). The results demonstrated that quinoa protein can produce foam with high stability, which increases its potential use in food processing. Using egg albumin (an outstanding foaming agent) as a reference, the foaming capacity and stability of egg albumin ranged from 156 to 200% and from 33 to 54% respectively.

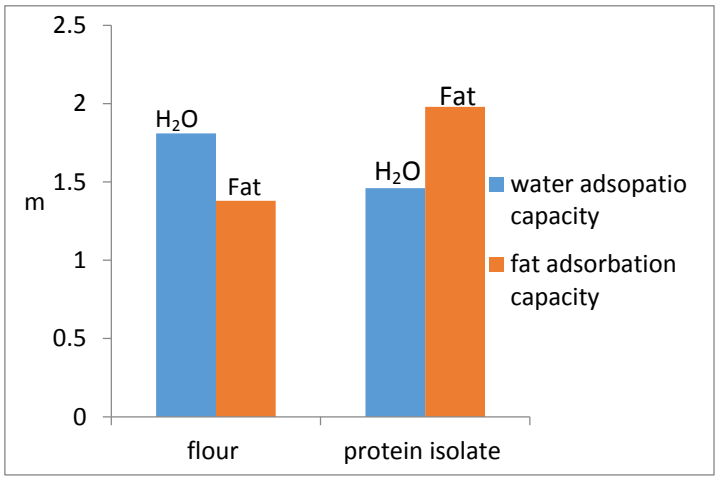

Fig.4. Water and fat absorption capacities of quinoa seed flour and protein isolates

Table (5): Emulsion properties of defatted quinoa flour and protein isolates.

Concentration of protein in sample	Samples		Means[2]
EAI	Quinoa flour	Protein isolate	
0.1%	0.8 ± 0.1	0.73 ± 0.56	0.312[d]
`0.5%	0.95 ± 0.5	1.3 ± 0.1	1.12[c]
1%	1.1 ± 0.1	2.3 ± 0.2	1.7[b]
3	3.03 ± 0.15	3.66 ± 0.32	3.35[a]
Means[1]	1.47[b]	2[a]	
ESI			
0.1%	33.5 ± 0.5	41.7 ± 0.25	37.63[c]
0.5%	36.33 ± 0.30	46.23 ± 0.49	41.28[a]
1%	34.1 ± 0.41	43.96 ± 0.45	39.05[b]
3	21.9 ± 0.90	29.75 ± 0.69	25.84[d]
Means[1]	31.46[b]	40.43[a]	

Means[1] in the same row with different letters are significant at ($p \leq 0.05$). LSD = 0.659 for different concentration
Means[2] in the same column with different letters are significant at ($p \leq 0.05$). LSD = 0.446 for quinoa and protein isolates

Table (6): Foaming capacity (%) of quinoa flour Proteins and protein Isolates at different concentration

Concentration (%)	Flour	Protein	Mean[2]
0.10	19.46±0.05	55.05±0.96	37.26[e]
0.50	24.19±0.73	65.13±0.99	44.67[d]
1.00	25.33±0.3	73.09±0.79	49.21[c]
2.00	29.3±0.26	76.45±0.5	52.88[b]
3	35.1±0.4	78.25±0.20	56.67[a]
Mean[1]	26.88[b]	69.59[a]	

Means[1] in the same row with different letters are significant at ($p \leq 0.05$). LSD = 0.48 for quinoa and protein isolates
Means[2] in the same column with different letters are significant at ($p \leq 0.05$).
LSD = 0.76 for different concentration

Foaming stability

Foaming stability for defatted quinoa flour and isolated protein is found in Fig. 5. From the data, we found that quinoa protein has high foam stability within 60 min higher than defatted quinoa flour and the foam stability gradually decreases within 60 min from 16.03 at zero time to 6.88 at 60min (ml/gdb).

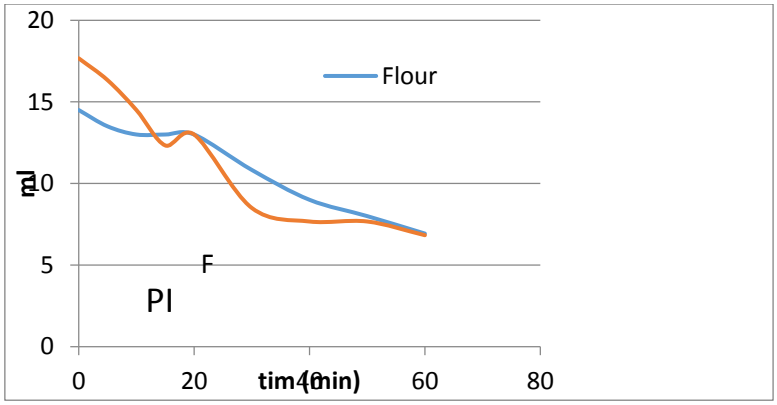

Fig. 5. foaming stability (ml) of quinoa flour Proteins and protein Isolates during 60 min.

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الخصائص الوظيفية والتغذوية لدقيق الكينوا ومعزول البروتين

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الملخص العربي

كان الهدف من هذه الدراسة هو تحديد التركيب الكيميائي، ومعرفة الأحماض الأمينية، والأحماض الأمينية الحدية، Polyacrylamide Gel Electrophoresis (SDS-PAGE)، وقابلية الهضم في المعمل ، والخصائص الوظيفية لدقيق الكينوا ومعزول البروتين. يحتوي QF على كميات كبيرة من البروتين والدهون والألياف والرماد والكربوهيدرات ١٤.٢٥ و ٧.٠٠ و ٥.١٤ و ٣.١٦ و ٧٠.٤٥٪ على التوالي. بلغت نسبة نقاوة البروتين المعزول الي ٩٤.١٢٪، والشوائب حوالي ٦٪. أول حمض أميني محدود في QF هو السيستين، والثاني هو الميثيونين، والثالث هو البرولين. في المقابل، في PI الحمض الأميني الأول هو السيستين، والثاني هو الهيستيدين، والثالث هو الميثيونين. كفاءة هضم بروتينات دقيق الكينوا QFP أعلى من البروتين المعزول PI. الوزن الجزيئي لبروتين دقيق الكينوا والبروتين المعزول متماثلة وهي (250,130, 100, 70, 55, 35, and 25 K Da). أظهر QFP قابلية هضم أعلى من PI . وكانت قدرة امتصاص المياه للدقيق أعلي من البروتين المعزول . كانت سعة امتصاص الدهون ١.٣٨ جم زيت/جم من الدقيق لدقيق الكينوا المشوه و ١.٩٨ جم زيت/جرام من عزل البروتين. بروتين الكينوا له ثبات رغوة عالي في غضون ٦٠ دقيقة.

الخلاصة

يمكن استخدام دقيق الكينوا ومعزول البروتين في منتجات المخابز كمصدر للبروتين لرفع القيمة الغذائية وأيضا الاستفادة من خصائصها الوظيفية وتحسين خواص الجودة للمنتجات المصنعة.