Annals of Agricultural Science (2015) 60(2), 297–305

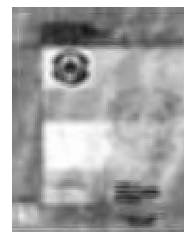
HOSTED BY

ELSEVIER

Faculty of Agriculture, Ain Shams University

Annals of Agricultural Science

www.elsevier.com/locate/aoas



Physicochemical and functional properties of quinoa protein isolate

S.A. Elsohaimy [a,*], **T.M. Refaay** [a], **M.A.M. Zaytoun** [b]

[a] *Food Technology Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt*
[b] *Food Science and Technology Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt*

Received 2 September 2015; accepted 13 October 2015
Available online 17 December 2015

KEYWORDS
Quality of quinoa protein;
Protein isolate;
Functional properties

Abstract This study was focused on the optimum conditions for preparing the protein isolate of quinoa seeds and investigates the physicochemical and functional properties of the isolated protein to assess the potential use of quinoa protein isolate in food applications and manufacturing. The protein isolate of quinoa was obtained by protein solubility at alkaline pH value (10), followed by precipitation at an acidic pH value (4.5). SDS–PAGE showed protein bands with 55 KDa corresponding to globulin and 31–33 KDa corresponding to chenoprotein in all extraction pHs. Quinoa protein had reasonable concentrations of essential amino acids (except tryptophan) with a high level of lysine (17.13%). A sharp minimum solubility was observed at the pH value (4.5), and the maximum value was observed at the alkaline pH value (10) ($P > 0.05$). Quinoa protein showed a high *In Vitro* digestibility (78.37 ± 1.08%). The quinoa protein showed water absorption (3.94 ± 0.06 ml/g) and (1.88 ± 0.02 ml/g) oil absorption. The foaming capacity of quinoa protein isolate was (69.28 ± 9.39% in average) and the foaming capacity was increased with the increase in the protein concentration. Quinoa protein isolate registered 54.54 ± 15.31% foam stability after 60 min. Emulsion ability index was ranged from 1.24 ± 0.05 m^2/g for 0.1% protein suspension to 3.38 ± 0.31 m^2/g for 3% protein suspension with average 2.10 ± 0.99 m^2/g. The average of emulsion stability index was (38.43 ± 7.22 min). Quinoa protein isolate is a promising and impressive nutritive source, which is leading to candidate it as a food supplement and functional food but still needs more advanced research to improve and proof its functional properties to be convenient for using in food processing and additives.

© 2015 Production and hosting by Elsevier B.V. on behalf of Faculty of Agriculture, Ain Shams University.

Introduction

Chenopodium quinoa Wild from the Chenopodiaceae family is a pseudocereal cultivated since ancient times by the Incas, and the FAO considers it as a perfect food (FAO, 1985). The seeds are an excellent example of functional food, defined as lowering

* Corresponding author. Tel.: +20 34593420; fax: +20 34593423.
E-mail address: elsohaimys@gmail.com (S.A. Elsohaimy).

Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

http://dx.doi.org/10.1016/j.aoas.2015.10.007
0570-1783 © 2015 Production and hosting by Elsevier B.V. on behalf of Faculty of Agriculture, Ain Shams University.

the risk of various diseases and exerting health-promoting effects (Repo-Carrasco et al., 2003; Vega-Galvez et al., 2010). Quinoa has been selected by FAO (2014) as one of the crops destined to offer food security in the 21st century, because the quinoa plants are tolerant to salinity and drought stress, and can grow on marginal regions (Jacobsen et al., 2003). Quinoa seeds have a high protein content (about 15%), and its essential amino acid balance is excellent, because of a wider amino acid spectrum than cereals and legumes (Ruales and Nair, 1992), with higher lysine (5.1–6.4%) and methionine (0.4–1.0%) contents (Bhargava et al., 2003). Quinoa contains lysine, methionine and cysteine higher than common cereals and legumes making it complementary to these crops. Quinoa's protein quantity ranged from 10.4% to 17.0% depending on its variety (Montaño Reyes et al., 2006). Besides its high content of protein, quinoa is known more for its protein quality (Repo-Carrasco et al., 2003). In contrast to quinoa, most grains are low in essential amino acid lysine, while most legumes and quinoa are low in sulfuric amino acids methionine and cysteine (Koziol, 1992). Protein for food not only provides nutrition but also should possess unique functional properties to facilitate processing and to develop the product. Functional properties of proteins are connected to the physicochemical properties, which govern the behavior of protein in foods. Emulsifying capacity (EC) and emulsion stability (ES) are two important functional characteristics of proteins that affect the behavior of various industrial products, including adhesives, cosmetics and packing material (Httiarachy and Kalapathy, 1998). Emulsion capacity and stability are critical parameters that affect the choice of protein for use in an industrial process (Wagner and Guenuen, 1999). Proteins can reduce tension in the water–oil interface and help prevent coalescence (McWatters and Cherry, 1982). A protein's stabilizing effect in an emulsion comes from the membrane matrix that surrounds the oil drop and prevents its coalescence (Jones, 1982). Quinoa is one of the most nutritive grains used as human food, and FAO has selected it as one of the crops destined to offer food security in this century (FAO, 1998). The digestibility of quinoa protein is the limiting factor in the protein utilization in food (de Romana et al., 1981). In vitro digestibility of quinoa protein varied from 76.3% to 80.5% (Repo-Carrasco-Valencia and Serna, 2011). The aim of the present study was to evaluate the physicochemical and functional properties of isolated protein from quinoa seeds to prove its nutritional quality and the ability its use as a food supplement.

Material and methods

Quinoa seeds (Chenopodium quinoa) were obtained from the Egyptian Company for Natural Oils, Cairo, Egypt. The seeds were cleaned of impurities and foreign materials and stored in a dry place at room temperature (25 ± 2 °C) for further analysis.

Flour preparation

Whole seeds were washed with cold water 4–5 times or until there was no foam to remove saponins, then oven-dried at 45 ± 1 °C for 24 h or until being dry. The whole seeds were ground into flour using Miller (Proctor Silex model E160, UPC) with a sixty-mesh screen (Abugoch et al., 2008).

Methods

Proximate analysis of quinoa seeds

The analysis of moisture, ash, crude fiber, total protein and total lipids were carried out as described in AOAC (2000). The total nitrogen free Carbohydrate (NFE) was calculated by the difference {100 – (protein + lipids + fiber + ash)}.

Preparation of protein isolate

Solubilization. The obtained flour was defatted three times with chloroform:methanol (2:1), 1:10 w/v with shaking for 2 h (Folsh and Stenly, 1957) to remove lipids from the sample. Quinoa protein isolate was prepared according to Alsohaimy et al. (2007). Fifty gram of defatted quinoa flour was suspended in 1000 ml deionized distilled water (1:20 v/v), and the pH was adjusted from 5 to 10 using 0.1 N NaOH and 0.1 N HCl. The suspension was stirred for 1 h with maintaining the pH at the determined value to reach the maximum level of solubilization. The mixture was centrifuged at 6000*g* at 20 °C for 30 min by high-speed cooling centrifuge (model K241R, Pro-Research, Centurion Scientific Ltd, UK). The protein concentration was measured in the supernatant by (QuickStart Bradford Protein Assay Kit). The effect of stirring time on protein extractability was examined at different times (30, 45, 60, 75, 90, 105 and 120 min), and the effect of adding NaCl on protein extractability was studied with different concentrations (0.00, 0.1, 0.25, 0.5, 0.75 and 1 M) of NaCl.

Acid precipitation of solubilized protein. The supernatant was collected, and the pH value was adjusted to (3–3.5, 4, 4.5, 5 and 5.5) to precipitate the protein. The suspension was centrifuged at 10,000*g* at 4 °C for 45 min. The precipitate was collected, freeze-dried and stored at −20 for further use.

Amino acid analysis

Amino acid analysis was carried out using performance amino acid analyzer (AAA 400, INGOS Ltd. Czech Republic) according to Block et al. (1958) and Spackman et al. (1958). Protein isolate was weighed (100 mg) into a glass ampoule, 10 ml of 6 N HCl was added to the ampoule, and the contents were hydrolyzed in an oven at 110 °C for 24 h. Oxygen was expelled in the ampoule by passing nitrogen gas through it. The excess of HCl was then removed from 1 ml hydrolyzed under vacuum at 80 °C with the occasionally addition of distilled water, then evaporated to dryness. HCl free residue was dissolved in exact 2 ml of loading buffer (6.2 M, pH 2.2). The analysis was carried out with a gas flow rate of 0.5 ml/min at 60 °C, and the reproducibility was 3%. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein according the following equation.

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

In vitro protein digestibility

In vitro protein digestibility was carried out by the multi-enzymes method of Bodwell et al. (1980) and Carbonaro et al. (1997). Porcine pancreatic trypsin (type IX, 15 310 units/mg protein), bovine b pancreatic chymotrypsin (type

II, 48 units/mg of solid), porcine intestinal peptidase (P-7500, 115 units/g of solid) and bacterial protease (type XIV, 4.4 units/mg of solid) (Sigma Chemical Co.) were used for the enzymatic digestion. 63.8 mg of sample in 10 ml of distilled water was equilibrated at 37 °C; the pH was adjusted to 8.0 with 1 N NaOH. One ml of three enzyme solution in water (1.58 mg of trypsin, 3.65 mg of chymotrypsin and 0.45 mg of peptidase) was added to the protein sample and digestion was allowed to proceed for 10 min at 37 °C. After addition of 1 ml (1.48 mg) of protease solution the digestion was continued for 9 min at 55 °C. The pH value was noticed after a further 1 min at 37 °C and used to estimate the *In vitro* protein digestibility according the following equation:

$$Y = 234.84 - 22.56X$$

where Y is the *In vitro* digestibility of protein (%), and X is the pH of the suspension after 20 min digestion.

SDS–PAGE of quinoa protein

The protein profile of quinoa was carried out by Sodium Dodecyl Sulfate–PolyAcreylamaide Gel Electrophoresis (SDS–PAGE) according to (Laemmli, 1970) with 5% of stacking gel and 12% of separating gel. Samples (20 μl) were prepared from 500 μl protein solution were added to 1 ml buffer (distilled water, 0.5 M Tri–HCl pH 6.8, glycerol, 10% SDS, 1% bromophenol blue and β-mercaptoethanol) and heated at 98 °C for 10 min, then applied to the sample wells. The standard protein marker (broad range molecular weight, Bio-Rad Hercules, USA), which contained (118, 85, 47, 80, 36, 26 and 20 KDa) was used as molecular weight standard. Electrophoretic migration was monitored at constant current (14 mA/gel) for 1.5–2 h. Gel was fixed with fixing solution (water/methanol/acetic acid 700:200:100 ml) for 30 min and then stained with Coomassie Brilliant Blue R-250 for 1 h. The stained gel was destained by frequently changing the fixing solution until the excess stain disappeared.

Protein solubility

The solubility of quinoa protein isolate was studied at pH values ranging from 1.00 to 10.00. A suspension with 5% protein isolate was prepared. For a better solubilization, the suspensions were stirred for 1 h, at room temperature 25° ± 2 °C, using a magnetic stirrer at different pH values achieved. The pH values were adjusted with a 0.1 N HCl and 0.1 N NaOH solutions. The suspensions at different pH values were centrifuged at 6000*g* for 30 min at 20 °C. The total nitrogen was determined in the supernatant by Kjeldahl method to determine the total protein. Protein solubility curve was constructed by using the average values obtained for each considered pH value (Aluko and Yada, 1993).

Oil and water absorption of protein isolate

For determination of oil and water of quinoa protein isolate, the method of Sathe and Salunkhe (1981) was followed. One gram of the sample was mixed with 10 ml deionized distilled water for 30 s in the mixture (Beville-Platinum, Model BLR 50 s/b, China). The protein sample was then allowed to stand at room temperature (25° ± 2 °C) for 30 min, centrifuged at 7000*g* for 30 min and the volume of supernatant was noted in a 10 ml graduate cylinder. The same procedure was repeated to determine the oil absorption of protein. Results were expressed on a dry weight basis.

Foaming capacity and stability

The method described by Tsutsui (1988) and Shahidi et al. (1995) was used to determine the foaming properties of protein isolate. Twenty milliliters of dried protein isolate (0.1%, 0.5%, 1% and 3% w/v) were whipped by (Waring blender model HGBTWTS3, USA) at high speed of (16,000 rpm) to incorporate the air for 1 min. then transferred to 50 ml cylinder, the total volume was measured at 0, 0.5, 5, 10, 40 and 60 min after whipping. Foam ability was expressed as foam expansion at 0 min while foam stability was expressed as foam expansion during 60 min. Foam expansion was calculated according the following equation:

$$\text{Foam expansion } (\%) = (A - B/B) \times 100$$

where A = volume after whipping (ml) at different time and B = volume before whipping

Emulsion capacity and stability

The emulsion capacity and stability were determined according to the method of Pearce and Kinsella (1978). 10 ml of sunflower oil was added to 30 ml (0.1%, 0.5%, 1% and 3% w/v of protein suspension at pH 10) and homogenized with mechanical homogenizer (MZIP Model 114, China) for 1 min at the highest speed. A 50 μl portion of the emulsion was pipetted from the bottom of the container at 0 and 10 min after homogenization. 5 ml of 0.1% SDS was added, and the absorbance was measured at 500 nm. The absorption was measured immediately ($A0$) and after 10 min ($A10$). The emulsion activity index (EAI) and the emulsion stability index (ESI) were calculated according the following equation:

$$\text{EAI } (\text{m}^2/\text{g}) = (2 \times 2.303 \times A0) - (0.25 \times \text{ protein concentration})$$

where $A0$ = absorbance measured immediately after emulsion formation at 500 nm.

$$\text{ESI (min)} = A0 \times (\Delta t/\Delta A)$$

where $\Delta A = A0 - A10$ and $\Delta t = 10$ min.

Statistical analysis

The data were analyzed using CoStat version 6.4, CoHort Software, Monterey, CA, USA. One-Way-ANOVA analysis with $p \leqslant 0.05$ was performed to identify significant differences among all studies parameters. All experiments carried out in triplicates.

Results and discussion

Proximate analysis

The proximate analysis of quinoa seeds was shown in Table 1. The moisture content of quinoa seed was 9.68 ± 0.33, ash

Table 1 Proximate analysis of quinoa seeds (% dry weight basis).

Constituents	% Dry weight basis
Moisture	9.68 ± 0.33
Ash	2.97 ± 0.021
Crud fiber	4.06 ± 0.34
Crude protein (N × 6.25)	14.03 ± 0.25
Crud fat	6.79 ± 0.19
Carbohydrate (NFE)	72.15 ± 0.28

Values presented as mean of triplicates ± SD, $P < 0.05$.

content was 2.97 ± 0.021, fiber content was 4.06 ± 0.34, protein content was 14.03 ± 0.25, fat content was 6.79 ± 0.19 and carbohydrate content (NFE) was 72.15 ± 0.28. A proximate analysis in this study was evident the potentiality of quinoa seed as a super and functional food due to its content of an essential nutritional elements (protein, carbohydrate, fat, and fiber). The results of the present study approved the previous results that showed the quinoa seed contained 11.2% moisture, 13.5–15% crude protein, 9.5% crude fiber, 1.2% total ash but contained a carbohydrate about (58.3%) (Abugoch, 2009; Ogungbenle, 2003) which is less than the investigated seeds in this study (72.15%). The previous studies reported that, the mean protein content of quinoa seeds ranged from 12% to 23% (Abugoch et al., 2008; Gonzalez et al., 1989; Karyotis et al., 2003). Compared to cereal grains, the total protein content of quinoa seed flour is higher than that of barley (11%), rice (7.5%) and corn (13.4%) (Abugoch et al., 2008). The reported protein value is higher than groundnut (8.8–11.6%) and cowpea (8.8–12.1%) (Aremu et al., 2005). On the other hand, quinoa seeds contain relatively minor proteins compared to legume seeds (22.75–37.9%) reported by Ogungbenle (2006).

Protein extractability

pH extractability

Fig. 1 illustrates concentrations of quinoa protein extracted in different pH values. Quinoa protein isolate was prepared by extracting the protein at alkaline pH values from 5 to 10. The obtained data declared that the protein solubility was gradually increased with the increase in pH values ($P > 0.05$). However, maximum solubility of quinoa protein was obtained at alkaline pH (10) (267.35 ± 1.26) that indicated its high content of acidic amino acids, which tends to be ionized at alkaline pH values (Alsohaimy et al., 2007). The data in this work are consistent with the previous studies of Nienke Lindeboom (2005) and Goundan (1992) who studied the quinoa protein and declared that the most storage protein in quinoa seed was extracted at alkaline pH values.

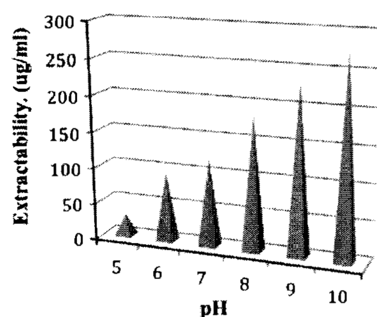


Fig. 1 Quinoa protein extraction at different pH values.

The effect of stirring period on extractability protein

The effect of stirring period on the protein extraction was studied and the results are shown in (Fig. 2). The extracted protein significantly increased with increasing the stirring period ($P < 0.05$) (Fig. 2). The stirring period has positive effect on the protein extraction. The maximum extractability power of protein was noted at 120 min.

The effect of adding NaCl on the protein extractability

The effect of adding NaCl salt in different concentrations (0.5–1 M) to the dissolving medium at the optimal pH value is shown in (Fig. 3). Adding NaCl to the extraction medium had significantly positive effect on the solubility of quinoa protein ($P > 0.05$). On the other hand there is no a significant differences in the extracted protein from 0.5 M to 1 M (from 467.58 to 496.98 μg/ml) compared to the differences from 0 to 0.5 M (from 353.61 to 467.58 μg/ml). Consequently, the considerable concentration might be added to enhance the protein extraction is 0.5 M NaCl. These findings are in agreement with Parakash (1986), who's reported that, the extractability of

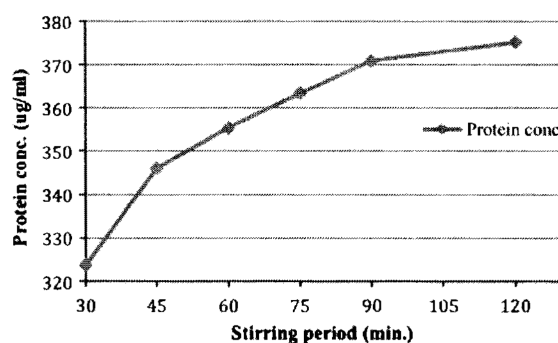


Fig. 2 The effect of stirring period (min) on protein extraction (μg/ml).

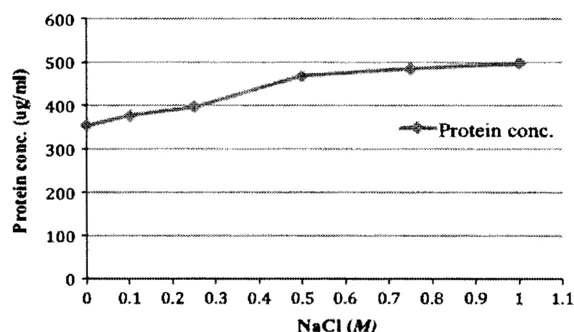


Fig. 3 The effect of adding NaCl on the extractability of quinoa protein.

protein from sesame seeds is increased with the increase in sodium chloride (NaCl) concentration from 0.05 to 2 M.

Acid precipitation of extracted proteins

The protein was precipitated from protein solution at different acidic pH values (4–6). The highest yield of precipitated protein was obtained at pH 4.5 (88.74 ± 0.53) (Fig. 4). The obtained results confirmed that, the isoelectric point of quinoa protein appeared near of the other proteins from different plant sources like legume proteins (pl = 4.5) (Alsohaimy et al., 2007), wheat proteins (pI = 4.22) (Payne and Corfield, 1979) and rice proteins (pI = 4.46) (Tanaka et al., 1980).

Amino acid composition

The amino acid composition of quinoa protein isolate was presented in (Table 2). Quinoa protein had a high level of lysine (17.13%), which registered the highest amino acid score (AAS) or chemical score (CS) (49.16), glutamic acid (12.80%) and aspartic acid (10.68%), while had a very low level of proline (0.10%) and arginine (0.03%). On the other hand quinoa protein had a moderate level of glycine (9.69%), alanine (5.34%) and phenylalanine (6.46%). From the obtained results appeared that the quinoa protein had reasonable concentrations of essential amino acids (except tryptophan) that are very important to human nutrition {threonine (1.47%), alanine (5.34%), valine (2.03%), methionine (1.70%), isoleucine (1.50%), leucine (4.60%), phenylalanine (6.46%) and histidine (2.76%)} (Table 2). Methionine and isoleucine are the limiting amino acids due to the lowest chemical score (1.00). Quinoa protein had lysine (17.13%) greater than wheat protein (2.6%) (Repo-Carrasco et al., 2003) and similar to soybean (18.32%) (Ranhotra, 1993). Other previous investigators had reported a high lysine content of quinoa (Ranhotra, 1993). Other than quinoa, most grains are low in the essential amino acid lysine, while most legumes are low in sulfuric amino acids methionine and cysteine (Koziol, 1992). Our results were in agreement with (Abugoch et al., 2008) who reported that essential amino acid levels in quinoa is similar to those of soybean and similar or high level of histidine and Ogungbenle et al. (2009) who reported that quinoa contains balanced essential amino acid than most cereals e.g. maize, millet, and sorghum. The obtained results declared that quinoa could serve as an excellent protein supplement.

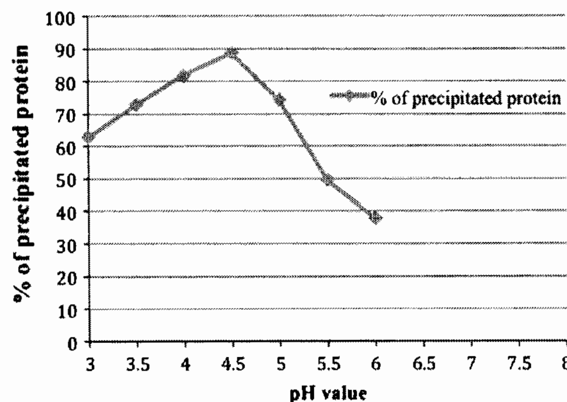


Fig. 4 Acid precipitation of extracted quinoa protein isolate.

In virto protein digestibility

The digestibility of proteins is an important parameter to evaluate their nutritional quality. Our experiment revealed that the *In vitro* digestibility of quinoa protein isolate was 78.37 ± 1.08% (untabulated data). Our results consistent with the earlier studies that showed the *In vitro* digestibility of protein of four quinoa varieties was between 75.3% and 84% (Repo-Carrasco-Valencia and Serna, 2011; Zia-Ur-Rehman and Shah, 2001). The quinoa protein digestibility showed the *In vitro* digestibility higher than wheat, which is very common use in human nutrition worldwide. The *In vitro* protein digestibility of wheat grains in the previous studies was 47% (Booth and Moran, 1946), 54.87% (Căpriţă et al., 2012) and 59% (Chick et al., 1947). The high digestibility of quinoa protein (78.37 ± 1.08%) is supporting its easy digest in the human stomach and consequently its health benefits for the human.

Electrophoresis patterns of quinoa protein

The electrophoresis patterns of quinoa-extracted protein in an aqueous extract with different pHs were shown in (Fig. 5). The protein with MW 85 KDa was not found in all pHs while the protein with 55 KDa corresponding to globulin according to Abugoch et al. (2008) was found in all pHs. The protein with

Table 2 Amino acid composition of quinoa protein isolate (g/100 g protein).

Essential amino acids	g/100 g	FAO/WHO/UNU pattern[a]	AAS (CS)	Non-essential amino acids	g/100 g	FAO/WHO/UNU pattern[a]
Histidine	2.76	1.6	1.73	Alanine	5.34	0.26
Leucine	4.60	1.9	2.42	Glycine	9.60	0.20
Isoleucine	1.30	1.3	1.00	Proline	0.10	0.61
Lysine	17.13	1.6	4.92	Serine	2.57	0.53
Methionine + cystine	1.70	1.7	1.00	Tyrosine	2.88	0.46
Phenylalanine + tyrosine	9.34	1.9	4.92	Glutamic	12.80	1.75
Threonine	1.47	0.9	1.63	Aspartic	8.54	0.88
Valine	2.03	1.8	1.13	Arginine	0.03	0.46
Tryptophan		0.5				

[a] Amino acids requirement pattern for adults, FAO/WHO/UNU (1985).

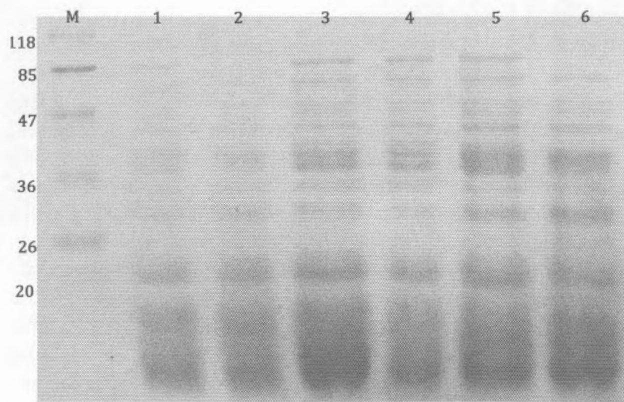


Fig. 5 SDS–Page of protein extractability on different pHs: Lane 1 – pH 5, Lane 2 – pH 6, Lane 3 – pH7, Lane 4 – pH 8, Lane 5 – pH 9, and Lane 6 – pH 10.

MW 33 KDa was found in all pHs with high expression. Proteins with 31–33 KDa corresponds to chenoprotein according to Abugoch et al. (2008). While protein with MW 22 KDa was found in all pH values but in high expression in pHs 7, 9 and 10. All protein bands less than 20 KDa corresponding to albumin components according to Brinegar et al. (1996) were found in all pH values with very high expression.

Proteins solubility

The solubility of quinoa protein isolate is related to the hydrophilic–hydrophobic balance of the proteins and the thermodynamics of its interaction with the solvent. The results presented in this study showed that the protein solubility is pH dependent (Fig. 6). The protein solubility was significantly increased with increasing pH value and reached the maximum solubility (75.21%) at pH 10 ($P > 0.05$). On the contrary, a sharp minimum solubility (25.59%) was observed at pH 4. The obtained data revealed that the major proteins of the isolated quinoa protein are acidic proteins and the optimum pH value for maximum protein solubility was at pH 10. The protein solubility profile is closely resembled to those already reported for several legumes and other cereal proteins by McWatters and Holmes (1979), Ganesh Kumar and Venkataraman (1980), Dench (1982) and Alsohaimy et al. (2007).

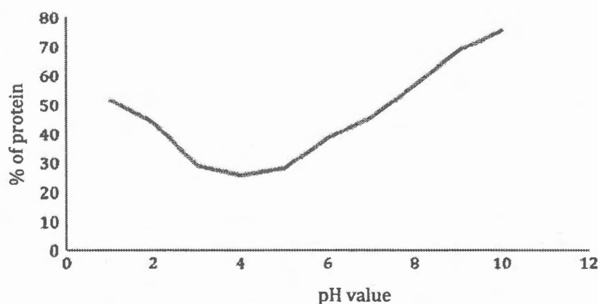


Fig. 6 Solubility of quinoa protein isolate.

Water and oil absorption

Water and oil absorption capacity of food materials is an important functional property because it improves mouthfeel and flavor retention. The quinoa protein showed water absorption 3.94 ± 0.06 ml/g protein that is slightly higher than wheat protein (3.67 ± 0.05 ml/g) and similar with soy protein, which is usually used in the human nutrition (4.05 ± 0.15 ml/g) (Ashraf et al., 2012) ($P < 0.05$). The same trend was observed with oil absorption where our results showed the oil absorption of quinoa protein 1.88 ± 0.02 ml/g while wheat protein was absorbed (1.58 ± 0.03 ml/g) and soy protein absorbed (2.10 ± 0.10 ml/g) (Ashraf et al., 2012). The good ability of quinoa protein isolates to absorb water and oil encourage the use it in bakery products to enhance their functional properties.

Foaming capacity and stability

Foaming capacity and stability are limiting factors in the characterization of the functional properties of proteins. Quinoa protein showed a high foaming capacity and stability. The foaming capacity of quinoa protein isolate was ranged from 58.37 ± 2.14% for 0.1% protein concentration to 78.62 ± 2.54% of 3% protein concentration with average (69.28%). Foaming capacity was significantly increased with the increase in protein concentration ($P < 0.05$). Foaming stability was ranged from 83.55 ± 5.95 in zero time to 54.54 ± 15.31% after 60 min ($P < 0.05$) (Table 3). The obtained results evidence the high ability of quinoa protein to make foam with high stability that is raising its potential for using in food processing. Considering an egg albumin (an excellent foaming agent) as a reference, the foaming capacity of egg albumin from literature was ranged from 156 to 200% and foaming capacity was ranged from 33% to 54% (Lomakina and Mikova, 2006). So, we can say that the quinoa protein had an ability to make foam less than egg albumin but showed foam stability similar to it. The ability of quinoa protein isolate to make foam might be referring to its content of different proteins as shown in protein profile (Fig. 5), which can contribute to the different functionalities. These findings were in contrast to those reported that the unfolding of protein during isoelectric precipitation, whereby the globular nature of the proteins was lost, did not increase their ability to form interfacial layers around air-bubbles (Aluko and Monu, 2003; Chauhan et al., 1999b). The foam stability of the quinoa protein products was similar and significantly higher than that of soybean protein, and lower than that of egg white protein (Abugoch et al., 2008). An explanation for this would be that; with the unfolding of the protein at low pH, the hydrophobic regions become exposed, and more binding to oil can occur. The improving foaming capacity will improve the functionality of quinoa protein isolate and support its use in bread baking process (Johnson et al., 1979; Ogungbenle et al., 2009).

Emulsion capacity and stability

Emulsion properties are the important functional properties of proteins that affect the behavior of food products. The emulsion ability index (EAI) and emulsion stability index (ESI) were shown in (Table 4). EAI ranged from 1.24 ± 0.05 m^2/g

Table 3 Foaming capacity and stability of quinoa protein isolate.

Protein conc.% (w/v)	Foaming capacity (%)	Foaming stability % at time interval (min)					
		0.00	0.5	5	10	40	60
0.10	58.37 ± 2.14	75.63 ± 1.65	61.35 ± 1.21	53.12 ± 1.34	45.13 ± 1.27	38.19 ± 1.57	34.83 ± 1.57
0.50	64.71 ± 1.79	82.57 ± 1.48	76.38 ± 1.36	66.47 ± 1.54	61.48 ± 1.36	54.43 ± 1.62	50.18 ± 1.85
1.00	75.41 ± 2.38	86.74 ± 1.76	81.45 ± 2.04	77.28 ± 1.23	71.68 ± 1.51	67.56 ± 2.15	64.75 ± 2.24
3.00	78.62 ± 2.54	89.24 ± 1.83	84.68 ± 1.36	79.59 ± 1.35	75.83 ± 1.53	71.64 ± 1.84	68.39 ± 1.68
Average	69.28 ± 9.39	**83.55 ± 5.95**	**75.97 ± 10.32**	**69.15 ± 12.10**	**63.53 ± 13.67**	**57.96 ± 15.08**	**54.54 ± 15.31**

Values presented in mean of triplicates ± SD, $P \leqslant 0.05$.

Table 4 Emulsion properties of quinoa protein isolate.

Con. (%)	EAI (m^2/g)	ESI (min)
0.10	1.24 ± 0.05	42.31 ± 0.58
0.50	1.39 ± 0.04	46.34 ± 1.24
1.00	2.37 ± 0.02	34.70 ± 1.31
3.00	3.38 ± 0.31	30.37 ± 0.97
Average	2.10 ± 0.99	38.43 ± 7.22

Values presented in mean of triplicates ± SD, $P \leqslant 0.05$.

for 0.1% protein suspension to 3.38 ± 0.31 m^2/g for 3% protein suspension with average 2.10 ± 0.99 m^2/g (Table 4). The EAI was significantly increased with increasing protein concentration with average 2.10 ± 0.99 m^2/g ($p < 0.05$). On the other hand, ESI ranged from 30.37 ± 0.97 to 46.34 ± 1.24 with average 38.43 ± 7.22. The ESI showed high values with 0.1–0.5% of protein and significantly decreased with increasing the protein concentration ($p < 0.05$) (Table 4). In comparison with bovine serum albumin as a reference, Hyun et al. (2003) showed that the emulsion activity index (EAI) of BSA was 54.4 m^2/g, and its stability ranged from 41.7 to 45 as pH dependent. It means that quinoa protein isolate has very low (EAI) 2.10 m^2/g compared to bovine serum albumin but has the (ESI) not so far from BSA. In the present study, quinoa protein isolate showed very low emulsion ability but good emulsion stability. Emulsion capacity and emulsion stability are critical parameters that affect the choice of the protein for use in an industrial process (McWatters and Cherry, 1982). Protein's stabilizing effect in an emulsion comes from the membrane matrix that surrounds the oil drop and prevents its coalescence (Jones, 1982). The emulsion properties of quinoa protein isolate need more advanced research to prove its functionality and use in food processing.

Conclusion

Quinoa is notable from FAO as a good source of quality protein and dietary fiber. Over more, a proximate analysis approved quinoa as a source of many nutrients like protein (which contained essential amino acids), fibers, fats, and carbohydrates. It might consume as a part of a balanced meal with many other food types to obtain overall good nutrition. Furthermore, Quinoa seeds contain proteins relatively less than legume seeds, but higher than other cereal grains like rice, wheat and barley. The optimum conditions for preparing protein isolate from quinoa seeds were noted in two steps: (a) protein extraction at alkaline pH value around (10) with stirring for 120 min and adding 0.5 M NaCl and (b) isoelectric point precipitation at pH value 4.5. The protein profile on SDS–PAGE gel showed the existence of globulin (55 KDa), chenoprotein (31–33 KDa) and albumin (less than 20 KDa). Quinoa protein had reasonable concentrations of essential amino acids (except tryptophan) that are very important to human nutrition. In the same time, quinoa protein had a very high level of lysine, while it contained a very low level of sulfur amino acids. Quinoa protein isolate showed a high solubility at alkaline pH values (10) and digestibility higher than other grains like wheat and rice. Holding capacity properties can modify the texture of dough or bakery products. Quinoa protein isolate showed a high foaming capacity and stability, with low emulsifying properties. Finally, we can conclude that the quinoa protein is a promising nutritive source and candidate for using as food supplement and functional food but still needs more advanced research to improve its functional properties to be suitable for using in food processing.

Acknowledgments

Authors are thankful to the Department of Food Technology, Arid Lands Cultivation Research Institute at City for Scientific Research and Technological Application, Alexandria, Egypt for providing the chemicals and facilities of this research work. The authors are thankful for the junior staff for their help in this work.

References

Abugoch, J.L.E., 2009. Quinoa (Chenopodium quinoa Willd.): composition, chemistry, nutritional, and functional properties. Adv. Food Nutr. Res. 58, 1–31.

Abugoch, L.E., Romero, N., Tapia, C.A., Silva, J., Rivera, M., 2008. Study of some physicochemical and functional properties of quinoa (Chenopodium quinoa Willd) protein isolates. J. Agric. Food Chem. 56 (12), 4745–4750.

Alsohaimy, S.A., Sitouhy, M.Z., El-Masry, R.A., 2007. Isolation and partial characterization of chickpea, lupine and lentil seed proteins. World J. Agric. Sci. 3 (1), 123–129.

Aluko, R.E., Monu, E., 2003. Functional and bioactive properties of quinoa seed protein hydrolysates. J. Food Sci. 68 (4), 1254–1258.

Aluko, R.E., Yada, R.Y., 1993. Relationship of hydrophobicity and solubility with some functional properties of cowpea (Vigna unguiculata) protein isolate. J. Sci. Food Agric. 62, 331–335.

AOAC, 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.

Aremu, M.O., Olaofe, O., Akintayo, E.T., 2005. Nutritional qualities assessment of the presence of hull in some Nigeria underutilized legume seeds. Bull. Pure Appl. Sci. 24, 47–52.

Ashraf, S., Saeed, S.M.G., Sayeed, S.A., Ali, R., 2012. Impact of microwave treatment on the functionality of cereals and legumes. Int. J. Agric. Biol. 14, 356–370.

Bhargava, A., Shukla, S., Ohri, D., 2003. Genetic variability and heritability of selected traits during different cuttings of vegetable Chenopodium. Indian J. Genet. Plant Breed. 63, 359–360.

Block, R.J., Durrum, E.L., Zweig, G., 1958. Annual of Paper Chromatography and Paper Electrophoresis, second ed. Academic Press, New York, USA, pp. 75–80.

Bodwell, C.E., Satterlee, L.D., Hackler, L.R., 1980. Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymic digestion methods. Am. J. Clin. Nutr. 33 (3), 677–686.

Booth, R.G., Moran, T., 1946. Digestibility of high extraction wheat in flours. Lancet 251, 119.

Brinegar, C., Sine, B., Nwokocha, L., 1996. High-Cysteine 2S seed storage proteins from quinoa (Chenopodium quinoa). J. Agric. Food Chem. 44, 1621–1623.

Căpriţă, Rodica, Căpriţă, Adrian, Creţescu, Nicu, Valeria, 2012. *In vitro* determination of wheat dry matter solubility and protein iuliana digestibility. Anim. Sci. Biotechnol. 45 (2), 113–116.

Carbonaro, M., Cappelloni, M., Nicoli, S., Lucarini, M., Carnovale, E., 1997. Solubility–digestibility relationship of legume proteins. J. Agric. Food Chem. 45, 3387–3394.

Chick, M., Cutting, M.E.M., Martin, C.I., Slack, E.B., 1947. Observations on the digestibility and nutritive value of the nitrogenous constituents of wheat bran. Br. J. Nutr. 1, 161.

de Romana, G. Lopez, Graham, G.G., Rojas, M., MacLean, W.C., 1981. Arch. Latinoam. Nutr. 31 (3), 485–497.

Dench, J.E., 1982. Extraction of nitrogenous material from winged bean (*Psophocarpus tetragonolobus* (L.) DC) flour and the preparation and properties of protein isolates. J. Sci. Food Agric. 33, 173–184.

Folsh, L.M., Stenly, G.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Food and Agriculture Organization (FAO), 1985. Food and Agriculture Organization of the United States/World Health Organization/United Nations University, Energy and Protein Requirements Report of a joint FAO/WHO/UNU meeting. World Health Organization, Geneva.

Food and Agriculture Organization (FAO), 1998. Underutilized Andean Food Crops. Rome.

Food and Agriculture Organization (FAO), 2014. Assessment of the international year of quinoa 2013. Hundred and forty-ninth session. CL 149/10. Rome, Italy.

Ganesh Kumar, K., Venkataraman, L.V., 1980. Chickpea seed proteins: isolation and characterization of 10.3S protein. J. Agric. Food Chem. 28, 524–529.

Gonzalez, J., Roldan, A., Gallardo, T., Prado, F., 1989. Quantitative determinations of chemical compounds with nutritional value from INCA crop: Chenopodium quinoa. Plant Foods Hum. Nutr. 39, 331–337.

Goundan, Saraswathi, 1992. Isolation and characterization of chenopodin, the major seed storage protein of quinoa (chenopodium quinoa). M.Sc. thesis. San Jose State University, California, USA.

Httiarachy, N.S., Kalapathy, U., 1998. Functional properties of soy proteins. In: Whitaker, J.R., Shahidi, F., Lopez-Munguia, A., Yada, R.Y., Fuller, G. (Eds.), Functional Properties of Proteins and Lipids. Washington, DC, USA, pp. 80–95.

Hyun, D.H., Lee, M., Halliwell, B., Jenner, P., 2003. Proteasomal inhibition causes the formation of protein aggregates containing a wide range of proteins, including nitrated proteins. J. Neurochem. 86, 363–367.

Jacobsen, S.E., Mujica, A., Jensen, C.R., 2003. The resistance of quinoa (Chenopodium quinoaWilld.) to adverse abiotic factors. Food Rev. Int. 19, 99–109.

Johnson, L.A., Havel, E.F., Hoseney, R.C., 1979. Bovine plasma as a replacement for egg in cakes. Cereal Chem. 56, 339–343.

Jones, K.W., 1982. Collagen properties in processed meat. Proc. Meat Ind. Res. Conf., 18–28

Karyotis, T., Iliadis, C., Noulas, C., Mitsibonas, T., 2003. Preliminary research on seed production and nutrient content for certain quinoa varieties in a saline–sodic. Soil J. Agron. Crop Sci. 189, 402–408.

Koziol, M., 1992. Chemical composition and nutritional evaluation of quinoa (Chenopodium quinoa Willd.). J. Food Compos. Anal. 5, 35–68.

Lindeboom, Nienke, 2005. Studies on the characterization, biosynthesis and isolation of starch and protein from quinoa (Chenopodium, quinoa wild). PhD Thesis, Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon.

Lomakina, K., Mikova, K., 2006. A study of the factors affecting the foaming properties of egg white – a review. Czech J. Food Sci. 24, 110–118.

McWatters, K.H., Cherry, J.P., 1982. Potential food uses of peanut seed proteins. In: Pattee, H.E., Young, C.T. (Eds.), Peanut Science and Technology. American Peanut Research and Education Society, Yakum, TX, USA, pp. 689–736.

McWatters, K.H., Holmes, M.R., 1979. Influence of moist heat on solubility and emulsification properties of soy and peanut flours. J. Food Sci. 44 (3), 774–776.

Montaño Reyes, E.A., Ávila Torres, D.P., Guevara Pulido, J.O., 2006. Componente nutricional de diferentes variedades de quinua de la región Andina. Avances Investigación en Ingeniería 5, 86–97.

Ogungbenle, H.N., 2003. Nutritional evaluation and functional properties of quinoa (Chenopodium quinoa) flour. Int. J. Food Sci. Nutr. 54 (2), 153–158.

Ogungbenle, H.N., 2006. Chemical composition functional properties and amino acid composition of some edible seeds. Riv. Italia Sos. Grasse 83, 74–79.

Ogungbenle, H.N., Oshodi, A.A., Oladimeji, M.O., 2009. The proximate and effect of salt applications on some functional properties of quinoa (Chenopodium quinoa) flour. Pak. J. Nutr. 8, 49–52.

Parakash, V., 1986. The effect of sodium chloride on the extractability of proteins from sesame seeds (Sesame indicum L.). J. Agric. Food Chem. 34, 256–259.

Payne, Peter I., Corfield, Kathryn G., 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145, 83–88.

Pearce, K.N., Kinsella, J.E., 1978. Emulsifying properties of proteins: evaluation of a turbidimetric technique. J. Agric. Food Chem. 26, 716–723.

Ranhotra, G., 1993. Composition and protein nutritional quality of quinoa. Cereal Chem. 70, 303–305.

Repo-Carrasco, R., Espinoza, C., Jacobsen, S.E., 2003. Nutritional value and use of the Andean crops quinoa (Chenopodium quinoa Willd) and kañiwa (Chenopodium pallidicaule). Food Rev. Int. 19 (1 & 2), 179–189.

Repo-Carrasco-Valencia, Serna, Lesli Astuhuaman, 2011. Quinoa (Chenopodium Quinoa, Willd) as source of dietary fiber and other functional components. Food Sci. Technol. (Campinas) 31 (1), 225–230.

Ruales, J., Nair, B., 1992. Nutritional quality of the protein in Chenopodium quinoa, Willd) quinoa seeds. Plant Foods Hum. Nutr. 42, 1–11.

Sathe, S.K., Salunkhe, D.K., 1981. Functional properties of great northern bean (Phaseolus vulgaris) proteins: emulsion, foaming, viscosity and gelation properties. J. Food Sci. 46, 71–75.

Shahidi, F., Han, X.Q., Synowiecki, J., 1995. Production and characteristics of protein hydrolysates from capelin. Food Chem. 53, 285–293.

Spackman, D.H., Stein, E.H., Moore, S., 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30, 1190–1191.

Tanaka, Kunisuke, Sugimoto, Toshio, Ogawa, Masahiro, Kasai, Zenzaburo, 1980. Isolation and characterization of two types of protein bodies in the rice endosperm. Agric. Biol. Chem. 44 (7), 1633–1639.

Tsutsui, T., 1988. Functional properties of heat treated egg yolk low density lipoprotein. J. Food Sci. 53, 1103–1106.

Vega-Galvez, A., Miranda, M., Vergana, J., Uribe, E., Puente, L., Martinez, E.A., 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willed.), an ancient Andean grain: a review. J. Sci. Food and Agric. 90 (15), 2541–2547.

Wagner, J., Guenuen, J., 1999. Surface functional properties of native, acid treated and reduced soy glycinin. 2. Emulsifying properties. J. Agric. Food Chem. 47, 2181–2187.

Zia-Ur-Rehman, Shah, W., 2001. Tannin contents and protein digestibility of black grams (Vigna mungo) after soaking and cooking. Plant Foods Hum. Nutr. 56, 265–273.