Pak. J. Bot., 57(6): DOI: http://dx.doi.org/10.30848/PJB2025-6(32)

PROTEIN PROFILING AND PROXIMATE ANALYSIS OF *CHENOPODIUM QUINOA* VARIETIES ADAPTED TO PAKISTAN

ANSA RANI[1], MUHAMMAD YASIN ASHRAF[2*], ISMAT UMAR[3], AHMED AKREM[4], SOHAIB MEHMOOD[3] AND BINISH KHALIQ[3*]

[1]*Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan*
[2]*Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan*
[3]*Department of Botany, University of Okara, Okara, Pakistan*
[4]*Institute of Botany, Bahauddin Zakariya University, Multan, Pakistan.*
[*]*Corresponding author's email: binish@uo.edu.pk ; niabmyashraf@gmail.com*

Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a seed crop native to the Andean region, known for its ability to grow in diverse agro-ecological conditions and its high nutritional value. Its seeds contain significant amounts of various micro- and macronutrients that are beneficial for both animal and human health. In the current study, protein profiling, quantification and proximate chemical composition were evaluated for food purposes. Five quinoa varieties (BLK, Q-8, Q-8/39, Q-11 and Q-13) selected for their favorable agronomic traits, were analyzed for biochemical composition and quality characteristics. The highest protein concentration (21.5 mg/g, 15.2 mg/g) was found in seed extract of varieties Q-8 and BLK at 25°C and 4°C, respectively. SDS-PAGE profiling of reduced seed extract displayed protein with molecular weight up to 150 kDa and 50 kDa at 25°C and 4°C, respectively. In non-reduced samples, the highest molecular weight observed was 70 kDa and 60 kDa at 25°C and 4°C, respectively. The highest values for protein content (13.9%), crude fiber (1.3%), crude fat (3.3%), ash content (1.95%), moisture content (10.5%) and carbohydrate content (66.5%) were observed across different varieties. The maximum carbohydrates content (65.9%) was found in Q-13, while BLK exhibited the lowest crude fiber content (1.1%) among the varieties studied. These findings provide valuable insights for the development of high-quality quinoa-based products.

Key words: *Chenopodium quinoa,* SDS-PAGE, Chemical composition, Protein, Carbohydrate.

Introduction

Quinoa (*Chenopodium quinoa* Willd) is a seed crop, and it is mainly cultivated in Andean region for many years. Its nutritive value is very high, so it is considered an important crop. It has become an interested crop worldwide because it can grow in different agro-ecological conditions (Jacobsen, 2011) and also being introduced for cultivation in different climatic region of England, Italy, Greece, and other European countries (Pulvento *et al.*, 2010). The global population is increasing at an alarming rate, along with metabolic diseases and climate change, therefore, food security and health are important issues. It is expected that in upcoming year's ecosystems will face increasing variation in climate and ratio of extreme events (Perez *et al.*, 2010). It is estimated that total global food demand will increase by approximately 50-60% between 2019 and 2050, driven by both population growth and rising per capita consumption (Falcon *et al.*, 2024). According to research, one out of nine individuals are facing problems of under nourishment (Anon., 2014). Quinoa can fulfill this need of food. Diabetes and metabolic disorders are also increasing day by day (Zimmet *et al.*, 2014). It is also estimated that the median age people of the world increased up to 26.8% in 2000 and will be increased up to 31.3% in 2050 (Lutz *et al.*, 2008), so, age related diseases like osteoporosis and cardiovascular diseases are also increasing (Lunenfeld & Stratton, 2013). To combat with metabolic disorders and diseases which are age related, food can play an important role in prevention and disease treatment. Food products which have special beneficial effects on human health are called functional food (Bigliardi & Galati, 2013).

Grain food or grain like food crops plays a great role in agriculture, currently 32 to 72% daily energy needs are derived from these crops (Poutanen *et al.*, 2014). Quinoa is grain-like crop which has been fulfilling the food requirements of Andean region from thousands of years but now it is being used worldwide. Quinoa has stress tolerant characteristics; more research work is required to explore its dietary composition to use it as food. Its global production can complete the demands of food of ever growing population of world in upcoming years. Quinoa is also being used in cosmetics, pharmaceuticals and in botanical supplements. It has balanced amount of essential amino acids e.g. lysine, lysine is one of the essential amino acids in most cereals. In quinoa lysine content is higher (27%) than corn, rice and wheat. Quinoa proteins also have histidine, and it is also higher than wheat and rice. Due to variation in climatic conditions amino acids contents varied and some essential amino acids like, lysine, tryptophan and tyrosine are found in quinoa (Gonelez *et al.*, 2012). As essential amino acids are present in quinoa at high level, so, it is considered as a plant which can provide all essential amino acids, which match to human nutritional standard which is stetted by FAO (Anon., 2013).

Quinoa's natural starch, characterized by consistently small granules measuring less than 3 µm in diameter, offers intriguing functional possibilities (Vega-Gálvez *et al.*, 2010). Notably, quinoa starch displays a low gelatinization temperature range (between 54-71◦C) and a relatively low enthalpy value (11 J g-1 starch). When compared to wheat and barley starch, quinoa stands out with its higher maximum viscosity, increased water absorption capacity, and superior swelling power (Filho *et al.*, 2017). Quinoa oil boasts a high content of essential fatty acids, including oleic (ranging from 19.7% to

29.5%), linoleic (from 49.0% to 56.4%), and linolenic (8.7% to 11.7%). Approximately 87% to 88% of the total fatty acids present in the seed are comprised of poly-unsaturated fatty acids (Angeli & Miguel, 2020; James, 2009). These compounds have gained significance due to their ability to promote various health advantages, such as enhancing the immune system, supporting cardiovascular health, aiding in cell membrane function, and increasing insulin sensitivity (Präger *et al.*, 2018).

Plants can be used for phytosteroid and quinoa seeds are best for this purpose because high level of phytosteroids is present in quinoa. There are many other known effects e.g. control of molt in insects and some hormones in mammals (Foucault *et al.*, 2012). Most common ecdysteroids is 20- hydroxyecdyson, which is present in many plants including quinoa. Among the 13-14 types of phytoecdysteroids identified in quinoa, 20-hydroxyecdyson is the most abundant and comprising of 63% to 92% present of the total phytoecdysteroid content (Graf *et al.*, 2015; Vidueiros *et al.*, 2015). Quinoa has a lot of health benefits for sports man, helps in improving their performance in that field, for diabetes patients, patients of anemia and for children. All these good features are due to the presence of vitamins, lot of fatty acids, minerals and quinoa's phytochemicals, making quinoa more beneficial than the other crops with respect to the human food and health (Vega galvez *et al.*, 2010). Keeping in view the importance of quinoa as a food crop, the current investigation was planned to study protein profiling, protein quantification and proximate analyses of different quinoa varieties. Specifically, the focused on locally adopted varieties cultivated in Pakistan including BLK, Q-8, Q-8/39, Q-11 and Q-13, which have shown promising agronomic performance under local agro-climatic conditions.

Materials and Methods

Plant material: For this study seeds of five varieties (BLK, Q-8, Q-8/39, Q-11, Q-13) of quinoa were collected from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Protein sample preparation: Seeds of five varieties (BLK, Q-11, Q-13, Q-8, and Q-8/39) of quinoa were ground separately in an electric blender. Seed powder one gram each of the five verities was taken in five Para film covered beakers separately with 10 ml phosphate buffer solution of pH 7. Then samples were stirred on magnetic stirrer (SCILOGEX-H550-S) for 3 to 4 hours at room temperature (25°C). Stirring was performed on 4°C as well. Ten stirred sample at 25°C and 4°C of all varieties were centrifuged at 5000 g for 40 minutes. Supernatants were filtered by using Whatman filter paper. 0.01% sodium azide (NaN_3) was added in each protein sample to store the samples for further processing. Bradford reagent 2 ml along with 40 µl of protein sample were taken in cuvette and absorbance was checked at 595 nm in spectrophotometer (UV/V spectrophotometer HALO SB-1 (Bradford, 1976).

Protein profile by SDS-PAGE: In all samples, protein content was assessed and integrated after running 12% SDS-PAGE (E-VS10-SYS, omniPAGE mini-System, Germany) according to (Laemmli, 1970). The general protocol was adopted for the preparation of 12% gel for the visualization of protein samples. Reduced and non-reduced dyes were used to visualize protein bands. Samples were mixed with 1:3 with both reduced and reduced dye. In case of reduced dye samples were heated at 95°C for 5 minutes before loading samples in gel wells to break the disulfide linkage present in the samples. Protein marker (Thermo Fisher Scientific, Cat # 26614) was used to check the mass of protein. Coomassie Brilliant Blue R-250 (CBB R-250) dye was used to stain gel.

Determination of proximate analysis

Moisture content: To determine the seed moisture contents AOCA (2000) method was followed for which three ground samples of each verity of *C. quinoa* (weighed 5g) were taken in China crucible and dried in hot air oven for 12 hours at 70°C and then immediately transferred into desiccators for cooling. Average weight loss of three samples were calculated, loss in weight showed the moisture content.

$$\text{Moisture content} = \frac{\text{Weight loss of maize (g)}}{\text{Weight of the original maize (g)}} \text{x } 100$$

Crude protein: The protein content in all the quinoa samples was studied according to the procedure of Association of Official Analytical Chemists (AOAC), 2000) using Kjeldahl apparatus. Weighed sample (1g) was digested with 50ml conc. sulphuric acid and 20 g digestion mixture (Copper.Sulphate ($CuSO_4$) and Potassium.Sulphate (KSO_4) in the ratio of 1:9) in Kjeldahl digestion flask until its color completely turned into blue. By adding water its volume changed into 500ml. 100 ml of this solution was taken in distillation flask and 400ml water and 200ml of 40% sodium hydroxide were added in it to neutralize the acid. And then distillation flask immediately fixed into condenser having a 500ml flask containing.40 ml of 4% boric acid. with mixed indicator and marked the Flask at 200ml. About 200ml of distillates having ammonium borate were collected and titrated against standard.0.1 N H_2SO_4.

$$\% \text{ Nitrogen} = \frac{\text{Volume of 0.01 } NH_2SO_4 \text{ used}}{\text{Weight of sample}} \text{ X } 0.0014 \text{ X } 100$$

$$\% \text{ Crude protein} = \quad \% \text{ Nitrogen X } 6.25$$

Crude.fat: To find crude fat, moisture free samples of each variety (5g) were poured into the cotton covered thimble (AOCA, 2000). Then the thimble was transferred in beakers containing Soxhlet assembly. Petroleum. Ether (40-60°C) was added in the flask having 1.5 times capacity of Soxhlet Assembly. For the circulation of cold water apparatus was fitted with condenser to tap. water. The apparatus was run for 18 hours at 60°. After the addition of ether, fatty constituents were dissolved in ether. After some time, the ether was evaporated left over fat was weighed.

$$\text{Crude fat \%} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \text{x } 100$$

Crude.fiber: Crude fibers were determined according to Association of Official Analytical Chemists (ACOIC, 2000) for which 200ml of 1.25 % sulphuric acid was taken in a beaker and 5g sample (moisture free) was added in it. After 30 minutes it was filtered by using muslin cloth and funnel. The residue left in muslin cloth was washed with hot water to make it acid free and then transferred into the beaker and 200ml of 1.25 % sodium hydroxide was added and again it was refluxed for 30 minutes. After 30 minutes it was filtered by using muslin cloth and residue were washed with hot water and transferred into pre weighed china crucible and dried in hot air oven at 130°C for 2 hours or until weight was recorded constant. Then, it was transferred into muffle furnace and loss in weight was recorded.

$$\text{Crude fiber \%} = \frac{\text{Weight of residue (g)} - \text{Weight of ash after ignition (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Ash content: Ash contents were analyzed by following the AOAC (2000) method, for which 5g ground seed sample was taken in china crucible, and it was put in the muffle furnace for 5 hours at 550°C. Then left over residue was weighed after cooling.

$$\text{Ash contents \%} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Carbohydrates content: By adding proximate, composition and subtracting from.100 carbohydrates contents were calculated according to AOAC (2000) method.

Statistical analysis: By using the analysis of variance and means, the data of proximate analysis of *C. quinoa* were examined and compared by Tukey's test using the software CoStat (Analytical Software, 2005).

Results

Protein quantification: Seeds of different varieties exhibited significantly ($p \leq 0.05$) different protein concentration at room (25°C) temperature and 4°C (Table 1). The highest protein content was observed (20.91 mg g^{-1}) in Q-13 and the lowest in Q-8/ 39 (18.4 mgg^{-1}) at room temperature (Fig. 1a). While at 4°C, the maximum values for protein contents (15.53 mg g^{-1}) were recorded in BLK and while minimum protein contents (14.2 mg g^{-1}) were found in Q-8/39. Quinoa variety Q-11 and Q-8 showed statistically similar protein concentration 14.24 mg g^{-1} and 14.5 mg g^{-1}rotein when extracted at 4°C (Fig. 1b).

Protein profiling: At 25°C, protein profiling of non-reduced samples was loaded in separate gel and their results were compared with protein marker which was loaded in lane M. In Q-11, Q-13, Q8/39 and BLk varieties same molecular weight of proteins bands. 40, 60 and 70 kDa were observed except in Q-8 where no protein bands were visible under non-reduced form as illustrated in (Fig. 2a). In reduced samples displayed that 40, 60, 70 and 150 kDa bands were visible in all varieties of quinoa (Fig. 2b). It is clear that seed extract at 25°C of all varieties showed same bands under reduced form. These results showed that quinoa varieties have different types of proteins, and these proteins also have disulphide linkage under the reduced form.

At 4°C, Varieties Q-11, Q-8, Q8/39 showed the different molecular weight of proteins i.e. 22, 35, 40, 60 and 70 kDa while varieties Q-11 and BLK showed no protein band under non-reduced form (Fig. 2c). Approximately 20, 30, 35, and 50 kDa protein bands were visible in the varieties Q-11, Q-13, Q-8, Q-8/39, and BLK under the reduced form. These results indicated that 70 kDa protein bands have disulphide linkage under reduced form and different types of proteins are present in all varieties of quinoa.

Proximate analysis: Results displayed that moisture % slightly varied in seeds of different varieties (Table 2). Moisture content varied non-significant among all the quinoa varieties however, the highest moisture contents were found in Q-8/39 (10.5%) statistically similar values were noted in, Q-11 (10.15%), Q-13 (10.10%), BLK (10.09%), and Q-8 (10.067) (Fig. 3a).

The current findings demonstrated significant variations in seed protein content among different varieties of quinoa (Table 2). The highest seed protein content was noted in variety BLK (14%) and minimum level of protein content was found in Q-13 (11.33%), closely followed by Q-8 (11.64%). Similarly, Q-11 (12.88%) and Q-8/39 (12.51%) had statistically similar values for protein contents (Fig. 3b).

Crude fat analysis of seeds of different quinoa varieties showed a significant variation (Table 2). The highest crude fat content was recorded for Q-13 (3.28%) which was statistically similar to that of Q-8/39 (2.94%) and Q-11 (2.85%) [Fig. 3c]. The quinoa variety BLK (2.13 %) contained minimum amount of crude fat which was at par to that of Q-8 (2.19%) [Fig. 3c].

The results for seed crude fibers analysis showed non-significant variations among all quinoa varieties (Table 2). However, the variety Q-13 (1.3%) had the maximum seed crude fibers contents (Fig. 3d) while the minimum seed crude fibers were obtained for BLK (1.1%) [Fig. 3d].

Table 1. Mean square values regarding analysis of variance showing temperature effect on protein extraction during quantification in different varieties of *C. quinoa* seeds.

Source	DF	Protein extraction at 25°C	Protein extraction at 0°C
Varieties	4	3.89078***	1.72537***
Error	10	0.00159	0.14114
Total	14	1.1128	0.59378

Note: *** Highly significant at $p \leq 0.05$

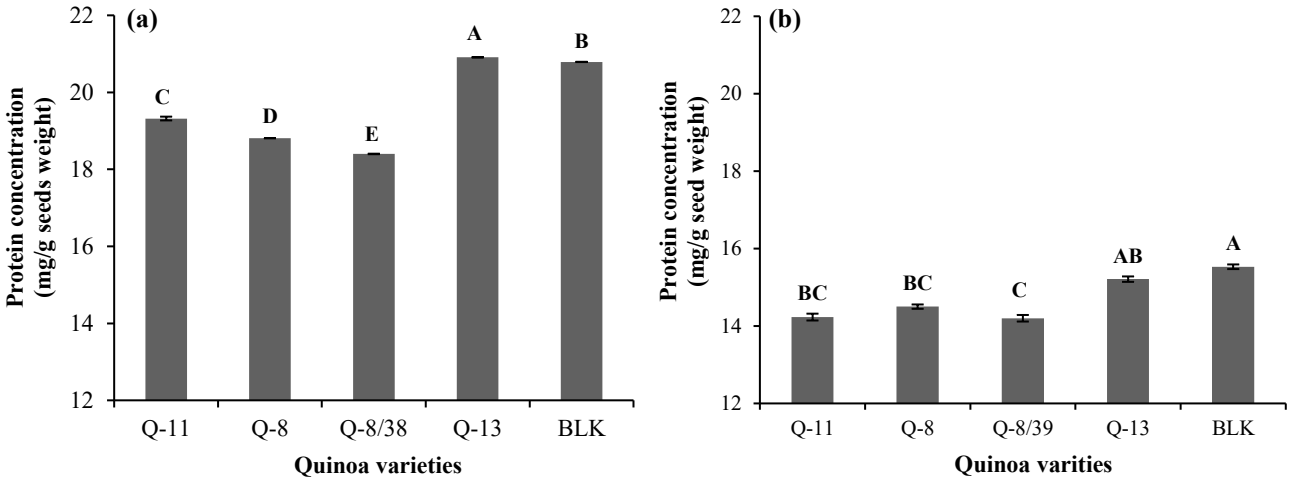

Fig. 1. Total protein concentration (mg/g) of five varieties of *C. quinoa* seeds: **a**, at 25°C; **b**, at 4°C. Different letters demonstrate significant differences among quinoa varieties.

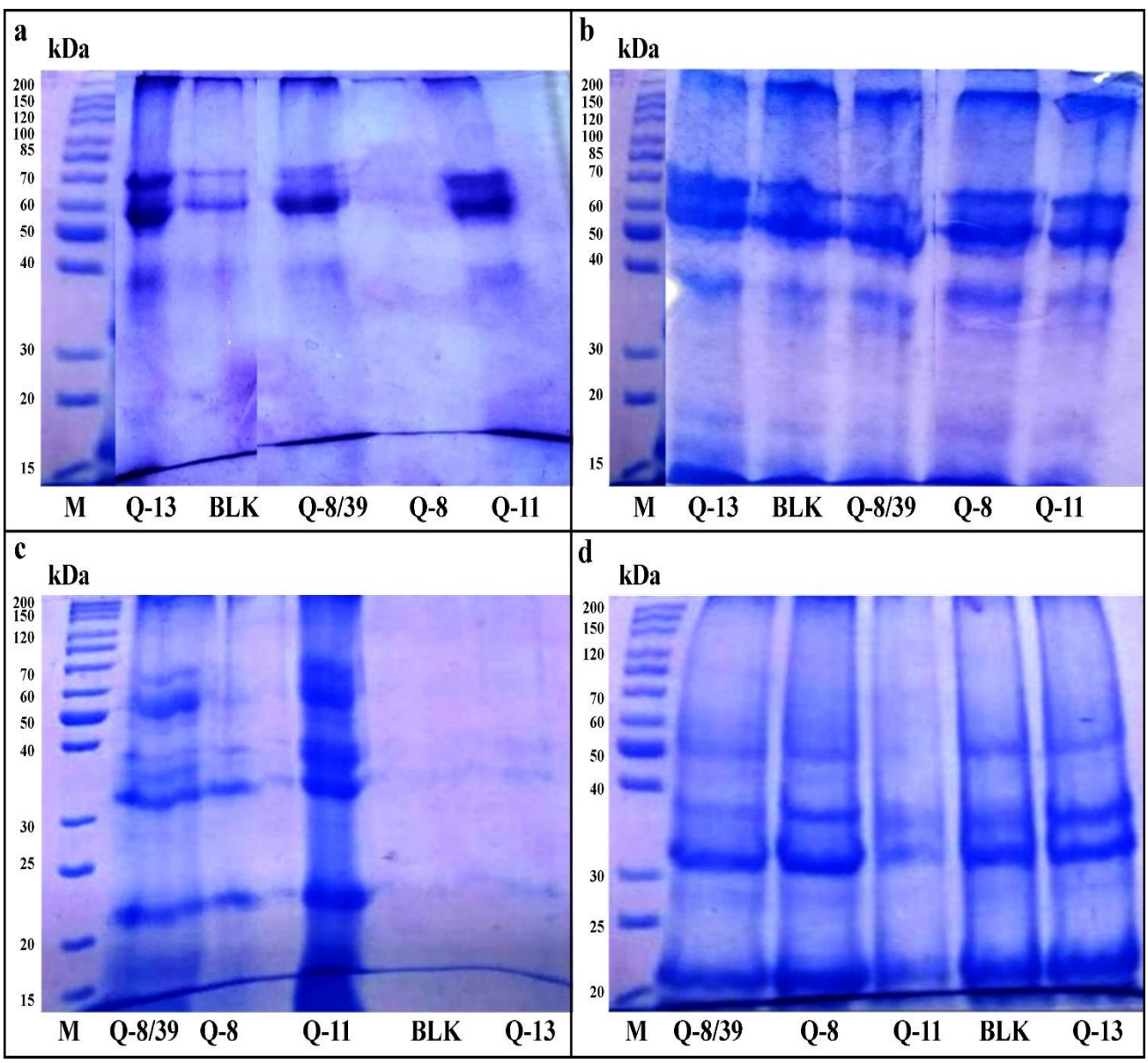

Fig. 2. Protein profile of different varieties of *C. quinoa* crude extract at 25°C and 4°C. (Lane M represents 5µl protein marker): a, represents non-reduced form at 25°C; b, represents reduced form at 25°C; c, represents non-reduced form at 4°C; d, represents reduced form at 4°C.

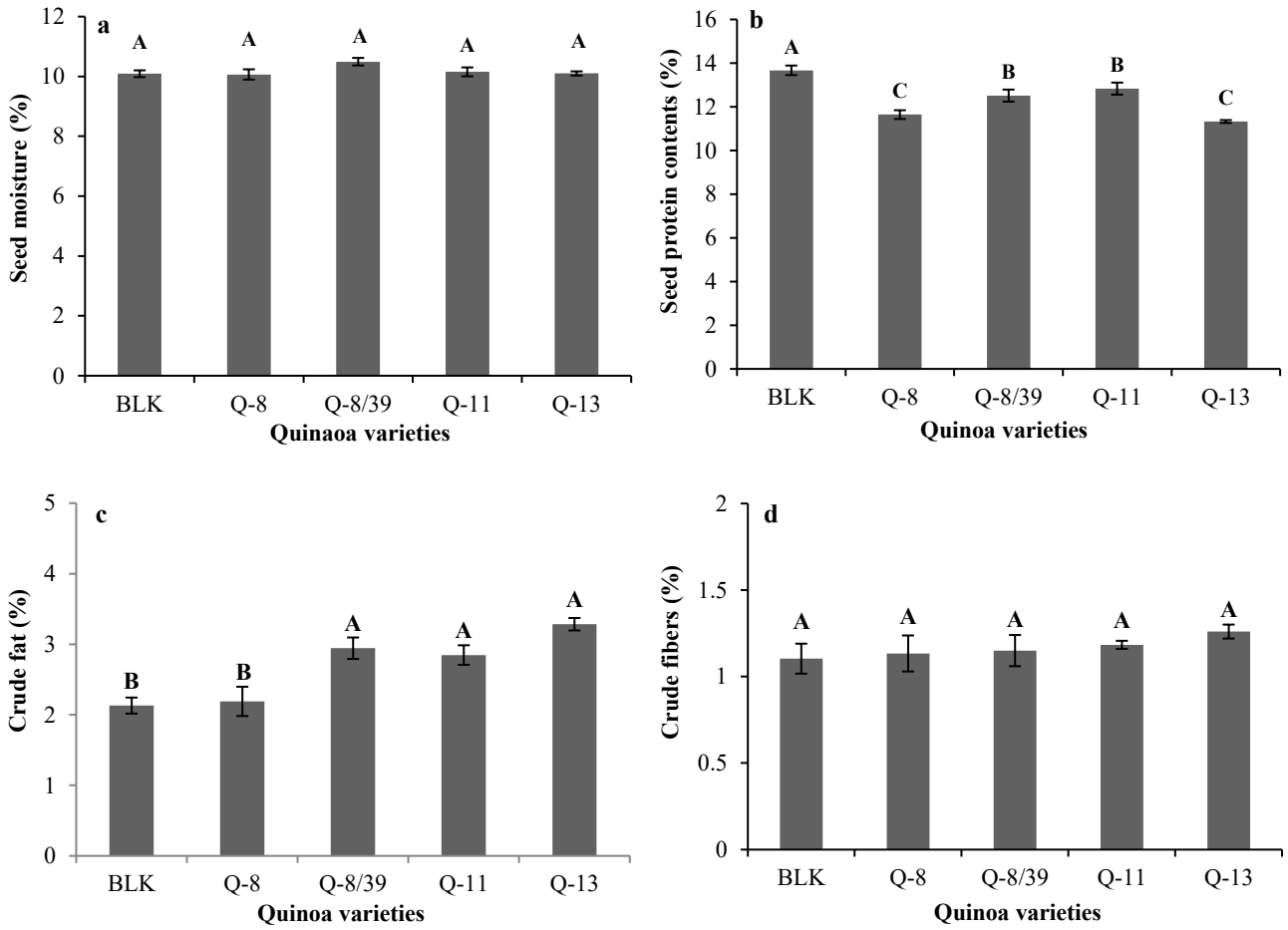

Fig. 3. **a**, moisture content; **b**, protein content; **c**, crude fat content; **d**, crude fiber contents in seed of different varieties of *C. quinoa.* Bars sharing similar letters show non-significant differences among quinoa varieties($p \leq 0.05$).

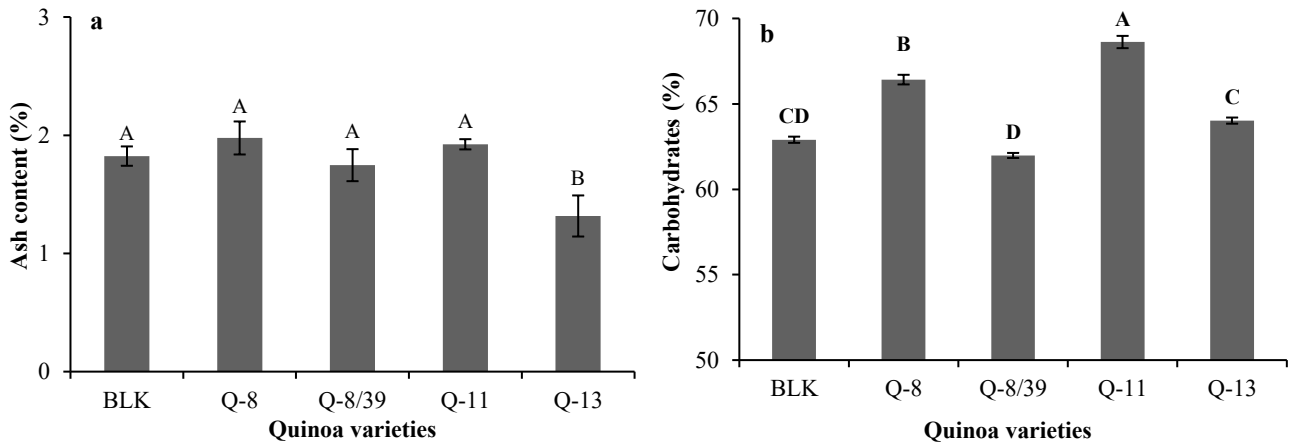

Fig. 4. **a**, ash content (%); **b**, content (%) of different varieties of *C. quinoa.* The data is represented as means ± SE of 3 replicates. Non-identical letters indicate significant difference among the varieties.

Table 2. Mean square values regarding analysis of variance showing variations in moisture, protein crude fat, crude fibers, ash and carbohydrates contents in different varieties of *C. quinoa* seeds.

S. variation	DF	Moisture (%)	Protein (%)	Crude fat (%)	Crude fibers (%)	Ash (%)	Carbohydrates (%)
Variety	4	0.9467^{NS}	2.65356**	0.75279**	0.01079^{NS}	0.20574*	22.0706***
Error	10	0.05104	0.14533	0.06366	0.01720	0.04599	0.2859
Total	14	0.06351	0.8619	0.26055	0.0154	0.09163	6.5101

Note: * = Significant; ** Highly significant at p≤0.05; *** = highly significant at p≤0.001; NS = Non-significant

Seed ash content analysis also indicated significant differences among all the quinoa varieties (Table 2). In Q-8 the values for ash content were the highest (1.98%), closely followed by Q-11 (1.92%), BLK (1.82%), and Q-8/39 (1.75%) [Fig. 4a]. Minimum ash content was noted for Q-13 (1.32%) [Fig. 4a].

The level of carbohydrate also exhibited significant differences (Table 2) among all the quinoa varieties used in current studies. The maximum carbohydrates contents were found in Q-11 (68.62%), followed by Q-8 (66.42%), > Q-13 (64.02%), > BLK (62.9%),> and Q-8/39 (61.98%) [Fig. 4b] The variations for carbohydrates contents among all the quinoa varieties were significant except Q-13 and BLK both have statistically similar values for this parameter and same was the case with Q-8/39 and BLK (Fig. 4b).

Discussion

In the current study protein profiling of seeds of *C. quinoa* was done and proximate chemical composition was also checked. This study was conducted on five different varieties of *C. quinoa*. During the experiment crude extract at 25ºC and 4ºC of seeds of all varieties was used to determine the molecular size of protein and seeds powder was used for proximate analyses (Fig. 1). Taking into account the obtained data, it was evident that in all quinoa samples carbohydrates were the major macronutrient, followed by protein and moisture content. Drzewiecki *et al*., (2003) reported that quinoa have protein content almost equal to cereal, but quinoa has high quality protein but resembling more with legume protein content (Valcárcel-Yamani & Lannes, 2012). Average protein in quinoa is 14% to16% (Hager *et al.*, 2012; Scanlin *et al.*, 2024), however, these values may be fluctuated in different verities from 7% to 20% (Bhargava *et al.*, 2007). Similarly in current study, average protein content ranges between 11 to 14 % in different varieties (Fig. 3b). Sulphur containing amino acid, lysine and arginine are essential amino acids, and quinoa has high level of these amino acids (Vega Glavz *et al.*, 2010). Quinoa has balanced amounts of essential amino acids e.g. lysine, which is one of the essential amino acids in most cereals (Parker-Gibson, 2015). In quinoa lysine content is higher than corn and wheat, it is almost 27% higher compared to rice. Quinoa proteins also have histidine, and it is also higher than wheat and rice (Dakhili *et al.*, 2019). Moreover, an adequate amount of aromatic amino acids are present in quinoa which can fulfill the need of children but these are in low quantity compared to wheat and rice (Hernández-Ledesma, 2019; Khaliq *et al.*, 2022). Variation in essential amino acids is present which is due to growing in different regions. Lysine, tryptophan and tyrosine found in quinoa are limiting amino acids (González *et al.*, 2012). The protein profiling present investigation is in line with the supported above findings (Fig. 2).

In the current findings, carbohydrates content ranged from 61- 69% (Fig. 4b) among various varieties of quinoa. There is a high percentage of maltose and D-xylose and low amount of glucose and fructose in quinoa flour which makes it ideal to use in malted drink formulations (Ogunbengle, 2003; Kohajdová *et al.*, 2023). A high content of minerals e.g calcium zinc and copper are present in quinoa (Repo-Carrasco *et al.*, 2003; Rybicka & Gliszczyńska-Świgło, 2017). Mineral (ash) concentration in quinoa is greater than most of the grain crops (Vega-Gálvez *et al.*, 2010). All of the above minerals present in quinoa is in balanced amount for human diet (Schlick & Bubenheim, 1996). Quinoa's starch granules to enzyme susceptibility were checked, such as isoamylase and β-amylase (Tang *et al.*, 2002), Porcine pancreatic α-amylase (Li *et al.*, 2016) at 38ºC has been reported. Complete hydrolysis of starch granules of *Chenopodium quinoa* after 30-31h reported by Tang *et al*., (2002). And their study shows that starch granules of quinoa are susceptible to enzyme digestion. Hydrolyses of starch is depend on structural characteristics and size of granules (Perez-Rea *et al.*, 2013).

Quinoa seeds are rich in a variety of vitamins and minerals, including noteworthy levels of calcium, iron, potassium, and magnesium, as highlighted in studies by Nowak *et al*., (2016) and Vilcacundo & Hernández-Ledesma (2017). Additionally, dietary fiber and bioactive compounds such as polyphenolic compounds are also present. Furthermore, the lipid content in quinoa is notably abundant in essential fatty acids like linoleic (Ω-6) and α-linolenic (Ω-3) polyunsaturated fatty acids (PUFA), aligning closely with the recommended Ω-6 to Ω-3 ratio for a healthy diet (5:1-10:1), according to Farinazzi-Machado *et al*., (2012). Although a healthy ratio of Ω-6 to Ω-3 is generally considered to be between 1/1 and 4/1, studies suggest that individuals adhering to a typical western diet may have a ratio ranging from 15/1 to nearly 17/1, as noted by Simopoulos (2006). The mean fat contents were not significantly different, spanning only from 2.1% to 3.3% in all the different varieties (Fig. 3c). The nutritional makeup of this seed has attracted attention from both producers and consumers, leading to numerous scientific studies exploring its exceptional nutritional value and diverse potential applications. For instance, Encina-Zelada *et al*., (2017) conducted research on the composition of various Peruvian samples of *Chenopodium quinoa* grains using Near-Infrared Transmission Spectroscopy. Their findings were generally comparable to the results obtained in the current study (Fig. 3).

The contents of fiber did not show much differences among different varieties (Fig. 3d). The study of Repo-Carrasco & Serna, (2011) demonstrates that these differences are not due to varieties but are true analytical differences. Our data aligns with previous literature findings (Alvarez-Jubete *et al.*, 2010) regarding the substantial unsaturation present in quinoa fat. In comparison to rice, quinoa exhibits an excess of 20 times more unsaturated fatty acids, particularly linoleic acid (C18:2). When juxtaposed with soybean, quinoa displays roughly half the amount of unsaturated fatty acids and a tenfold reduction in saturated fatty acids (Rao & Shahid, 2012). These variations can be attributed to the lower fat content in rice and the higher fat content in soybeans. The daily human diet can be bettered by addition of quinoa varieties in raw as well processed form as the quinoa is rich in fiber, protein, fat content and carbohydrate content.

Conclusion

Nutritional composition in quinoa seeds, with particular emphasis on protein assessment and characterization, was investigated on five quinoa varieties (BLK, Q-8, Q-8/39, Q-11 and Q-13). Various nutritionally significant compounds that could play a crucial role in promoting the consumption of this pseudo-cereal were identified. The highest protein and carbohydrates contents were found in seed extract of varieties Q-8 and Q-13 respectively, while ash content, moisture content, crude fat and crude fiber were also present in sufficient amounts to meet the nutritional requirements for human consumption.

References

Alvarez-Jubete, L., E.K. Arendt and E. Gallagher. 2010. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trend. Food Sci. Technol.*, 21(2): 106-113.

Angeli, V. and S.P. Miguel. 2020. An overview of the potentials of the "Golden Grain" and socio-economic and environmental aspects of its cultivation and marketization. *Foods*, 9(2): 216**.**

Anonymous. 2013. International year of quinoa: A future sown thousands of years ago.

Anonymous. 2014. Strengthening the enabling environment for food security and nutrition.

Bhargava, A., S. Shukla, S. Rajan and D. Ohri. 2007. Genetic diversity for morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.) germplasm. *Genet. Resour. Crop. Evol.*, 54(1): 167-173.

Bigliardi, B. and F. Galati. 2013. Innovation trends in the food industry: the case of functional foods. *Trend. Food Sci. Technol.*, 31(2): 118-129.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72(1-2): 248-254.

Dakhili, S., L. Abdolalizadeh, S.M. Hosseini, S. Shojaee-Aliabadi and L. Mirmoghtadaie. 2019. Quinoa protein: Composition, structure and functional properties. *Food Chem.*, 299: 125161.

Drzewiecki, J., E. Delgado-Licon, R. Haruenkit, E. Pawelzik, O. Martin-Belloso, Y.S. Park and S. Gorinstein. 2003. Identification and differences of total proteins and their soluble fractions in some pseudocereals based on electrophoretic patterns. *J. Agric. Food Chem.*, 51(26): 7798-7804.

Encina-Zelada, C., V. Cadavez, J. Pereda, L. Gómez-Pando, B. Salvá-Ruíz, J.A. Teixeira, I. Martha, H.L. Kristian and U. Gonzales-Barron. 2017. Estimation of composition of quinoa (*Chenopodium quinoa* Willd.) grains by Near-infrared transmission spectroscopy. *LWT-Food Sci. Technol.*, 79: 126-134.

Falcon, W.P., M. Rojas-Downing, C. Grovermann and J.W. Jones. 2024. The future of foods – sustainable food technology trends to 2050. *Sustainable Food Technology*, 3(FB): 14-35.

Farinazzi-Machado, F.M.V., S.M. Barbalho, M. Oshiiwa, R. Goulart and O.J. Pessan. 2012. Use of cereal bars with quinoa (*Chenopodium quinoa* W.) to reduce risk factors related to cardiovascular diseases. *J. Food Sci. Technol.*, 32: 239-244.

Filho, A.M.M., M.R. Pirozi, J.T.D.S. Borges, H.M. Pinheiro Sant'Ana, J.B.P. Chaves and J.S.D.R. Coimbra. 2017. Quinoa: Nutritional, functional, and antinutritional aspects. *Crit. Rev. Food Sci. Nutr.*, 57(8): 1618-1630.

Foucault, A.S., V. Mathé, R. Lafont, P. Even, W. Dioh, S. Veillet, T. Daniel, H. Jean-François, H. Dominique and A. Quignard-Boulangé. 2012. Quinoa extract enriched in 20-hydroxyecdysone protects mice from diet-induced obesity and modulates adipokines expression. *Obesity*, 20(2): 270-277.

González, J.A., Y. Konishi, M. Bruno, M. Valoy and F.E. Prado. 2012. Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (*Chenopodium quinoa*) cultivars from two different agroecological regions. *J. Sci. Food Agric.*, 92(6): 1222-1229.

Graf, B.L., P. Rojas-Silva, L.E. Rojo, J. Delatorre-Herrera, M.E. Baldeón and I. Raskin. 2015. Innovations in health value and functional food development of quinoa (*Chenopodium quinoa* Willd.). *Compr. Rev. Food Sci. Food Saf.*, 14(4): 431-445.

Hager, A.S., A. Wolter, F. Jacob, E. Zannini and E.K. Arendt. 2012. Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. *J. Cereal Sci.*, 56(2): 239-247.

Hernández-Ledesma, B. 2019. Quinoa (*Chenopodium quinoa* Willd.) as source of bioactive compounds: A review. *BCHD:* 2769-2426, 2(3): 27-47.

Jacobsen, S.E. 2011. The situation for quinoa and its production in southern Bolivia: from economic success to environmental disaster. *J. Agron. Crop. Sci.*, 197(5): 390-399.

James, L.E.A. 2009. Quinoa (*Chenopodium quinoa* Willd.): Composition, chemistry, nutritional, and functional properties. *Adv. Food Nutr. Res.*, 58: 1-31.

Khaliq, B., H. Sarwar, A. Akrem, M. Azam and N. Ali. 2022. Isolation of napin from *Brassica nigra* seeds and coagulation activity to turbid pond water. *Wat. Supply*, 22: 6050-6058.

Kohajdová, Z., T. Holkovičová, L. Minarovičová, M. Lauková, J. Hojerová, G. Greif and D. Ťažká. 2023. Potential of quinoa for production of new non-dairy beverages with reduced glycemic index. *JMBFS.*, 12(6): 9885-9885.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nat.*, 227(5259): 680-685.

Li, G., S. Wang and F. Zhu. 2016. Physicochemical properties of quinoa starch. *Carbohydr. Polym.*, 137: 328-338.

Lunenfeld, B. and P. Stratton. 2013. The clinical consequences of an ageing world and preventive strategies. *Best Pract. Res. Clin. Obstet. Gynaecol.*, 27(5): 643-659.

Lutz, W., W. Sanderson and S. Scherbov. 2008. The coming acceleration of global population ageing. *Nat.*, 451(7179): 716-719.

Nowak, V., J. Du and R.U. Charrondière. 2016. Assessment of the nutritional composition of quinoa (*Chenopodium quinoa* Willd.). *Food Chem.*, 193: 47-54.

Ogunbengle, H.N. 2003. Nutritional evaluation and functional properties of quinoa (*Chenopodium quinoa*) flour. *Int. J. Food Sci. Nutr.,* 54: 153-158.

Parker-Gibson, N. 2015. Quinoa: Catalyst or catastrophe? *J. Agric. Food Inf.*, 16(2): 113-122.

Perez, C., C. Nicklin, O. Dangles, S. Vanek, S.G. Sherwood, S. Halloy, K.A. Garrett and G.A. Forbes. 2010. Climate change in the high Andes: Implications and adaptation strategies for small-scale farmers. *Int. J. Environ. Cult. Econ. Soc. Sustain.*, 6: 71-88.

Perez-Rea, D., C. Rojas, S. Carballo, W. Aguilar, B. Bergenståhl and L. Nilsson. 2013. Enzymatic hydrolysis of *Canna indica*, *Manihot esculenta* and *Xanthosoma sagittifolium* native starches below the gelatinization temperature. *Starch/Stärke*, 65(1-2): 151-161.

Poutanen, K., N. Sozer and V.G. Della. 2014. How can technology help to deliver more of grain in cereal foods for a healthy diet? *J. Cereal Sci.*, 59(3): 327-336.

Präger, A., S. Munz, P.M. Nkebiwe, B. Mast and S. Graeff-Hönninger. 2018. Yield and quality characteristics of different quinoa (*Chenopodium quinoa* Willd.) cultivars grown under field conditions in Southwestern Germany. *J. Agron.,* 8(10): 197.

Pulvento, C., M. Riccardi, A. Lavini, R. d'Andria, G. Iafelice and E. Marconi. 2010. Field trial evaluation of two *Chenopodium quinoa* genotypes grown under rain-fed conditions in a typical Mediterranean environment in South Italy. *J. Agron. Crop Sci.*, 196(6): 407-411.

Rao, N.K. and M. Shahid. 2012. Quinoa-A promising new crop for the Arabian Peninsula. *AEJAES*., 12(10): 1350-1355.

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

Repo-Carrasco-Valencia, R.A.M. and L.A. Serna. 2011. Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber and other functional components. *J. Food Sci. Technol.*, 31: 225-230.

Rybicka, I. and A. Gliszczyńska-Świgło. 2017. Minerals in grain gluten-free products. The content of calcium, potassium, magnesium, sodium, copper, iron, manganese, and zinc. *J. Food Compost. Anal.*, 59: 61-67.

Scanlin, L., K.A. Lewis and P. Dugger. 2024. Chapter 19 - Quinoa as a sustainable protein source: Production, nutrition, and processing. In: *Sustainable Protein Sources* (2nd Edition). Academic Press, pp. 381-398.

Schlick, G. and D.L. Bubenheim. 1996. Quinoa: candidate crop for NASA's controlled ecological life support systems. *Prog. New Crop.*, 632-640.

Simopoulos, A.P. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed. Pharmacother.*, 60(9): 502-507.

Tang, H., K. Watanabe and T. Mitsunaga. 2002. Characterization of storage starches from quinoa, barley and adzuki seeds. *Carbohydr. Polym.*, 49(1): 13-22.

Valcárcel-Yamani, B. and S.D.S. Lannes. 2012. Applications of quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* spp.) and their influence in the nutritional value of cereal based foods. *Food Pub. Health*, 2(6): 265-275.

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

Vidueiros, S.M., R.N. Curti, L.M. Dyner, M.J. Binaghi, G. Peterson, H.D. Bertero and A.N. Pallaro. 2015. Diversity and interrelationships in nutritional traits in cultivated quinoa (*Chenopodium quinoa* Willd.) from Northwest Argentina. *J. Cereal Sci.*, 62: 87-93.

Vilcacundo, R. and B. Hernández-Ledesma. 2017. Nutritional and biological value of quinoa (*Chenopodium quinoa* Willd.). *Curr. Opin. Food Sci.*, 14: 1-6.

Zimmet, P.Z., D.J. Magliano, W.H. Herman and J.E. Shaw. 2014. Diabetes: A 21st century challenge. *Lancet Diabet. Endocrinol.,* 2(1): 56-64.

(Received for publication 15 August 2024)