INTRODUCTION

Plants being vital dietary source for humans contain commodious variety of bio active moieties. There is a dire need to validate their potential health benefits in human nutrition and provide valuable scientific information for not only end user but also for other scientific community (Lairon, 2010). Cereals and pseudocereals serve as staple food in many countries of the world. Among pseudocereals, quinoa (Chenopodium quinoa) is an annual dicotyledonous plant usually around 1-2 m high. Its seeds may be consumed as human food in flour, baked products and in animal feedstock for its higher nutritive worth (Repo-Carrasco-Valencia et al., 2010). Quinoa is becoming popular in Pakistan because of its ability to grow in drought and poor soil conditions (Basra et al., 2014).

The nutritional value of a food is determined by its protein quality, which depends mainly on its influence on anti-nutritional factors (Comai et al., 2007). Importance of the quinoa proteins is mainly due to its quality. Quinoa proteins belong to globulin and albumin, which have a balanced configuration of essential amino acids comparable to the configuration of casein protein (Ranhotra et al., 1993). The protein contents of quinoa range from 7 to 23% which is much higher than any other cereal crop (James and Lilian, 2009; Karyotis et al., 2003).

For the nutritional quality of proteins, the protein efficiency ratio (PER) or net protein utilization (NPU) are generally used as indicators. It have been reported that the protein efficiency ratio of the heated quinoa was 30%; higher than that of raw quinoa (Raina and Datta, 1992). Moreover, the quinoa protein quality is superior to most cereal grains and have potential to be used as protein concentrates in food industry. Nevertheless, the quinoa seed digestibility is the limiting aspect in energy and protein utilization, which can be significantly changed into better-quality by milling (Lopez de Romana et al., 1981).

Fat content of quinoa range from 4.4% to 8.8% with 55% to 63% of essential polyunsaturated fatty acids. Quinoa seed oil content is higher than corn (4.9%) but lower than soya (20.9%) (Wood et al., 1993). Quinoa fat is completely natural, un-processed and is best for the human body. It is especially rich in linolenic and linoleic acid (Omega-3) (Ruales and Nair, 1993) which are least harmful to heart and arteries. It has the right combination of fat and higher n-6/n-3 percentage (Alvarez-Jubete et al., 2009). Monounsaturated fatty acids contribute 25-28.7% (oleic acid), polyunsaturated is 58.3% (linoleic acid) and total saturated contribute 19-12.3% (palmitic acid) (Ryan et al., 2007). The relative abundance of monounsaturated fatty acids in quinoa is conducive to the promotion of good health. Keeping in view the above mentioned facts and figures, the current study has been designed to evaluate indigenous quinoa cultivars for their nutritional profile.

MATERIAL AND METHODS

Procurement of raw material: Four genotypes of quinoa named C. quinoa V7, C. quinoa V2, C. quinoa V9, C. quinoa V1, were procured from Department of Crop Physiology,
University of Agriculture, Faisalabad-Pakistan.

**Chemical analysis:** The quinoa flour samples were evaluated for chemical composition i.e. moisture, crude protein, crude fat, ash content and crude fiber according to their respective methods as described in AACC (2000).

**Determination of mineral content:** The samples were investigated for their mineral profile after wet digestion in di-acid mixture of HNO₃:HClO₄ by following the guidelines of AOAC (2006).

**Determination of fatty acid profile of quinoa:** Fatty acid profile of quinoa was determined by following the method as described by Przybylski et al. (1994) with some modifications. Oil from the quinoa was extracted through soxhlet extraction method by using hexane as solvent. Fatty acid methyl esters (FAME) were prepared from extracted oil by the method as described by Ryan et al. (2007). For Fatty acid methyl ester (FAME) analysis, a Column J and W DB-23 (60 m x 0.32 mm x 0.25μm) was used. The column was connected to a Gas chromatograph Shimadzu GC-17A equipped with a flame-ionization detector (FID). Helium was used as the carrier gas. The temperature was programmed as follows: Injection temperature, 250°C; detector temperature 285°C; Column temperature Start 100 °C, 10 °C/min to 180 °C, hold 5 min, 5°C to 240 °C hold 25 min. Auto Sampler set for 3 sample rinses followed after injection with 3 solvent rinses.

**Preparation of quinoa protein isolates:** Quinoa protein isolates were prepared according to the method described by Aludatt et al. (2012).

**Functional properties of quinoa protein isolates:** Functional properties of quinoa protein isolates such as water absorption capacity, oil absorption capacity and foaming capacity (FC) were determined according to their respective procedures, described by Tomotake et al. (2002) and Sze-Tao and Sathe (2000).

**In-vitro quinoa protein digestion:** In-vitro quinoa protein digestion was determined by following the method as described by Tinus et al. (2012). Milled quinoa equivalent to 62.5 mg protein was weighed and rehydrated in 10 mL of water at 37°C for 1 h, after which the pH was adjusted to about 8.0 with 0.1 M NaOH and/or HCl. Ten millilitres (10 mL) of a multi-enzyme solution was prepared consisting of about 16 mg of trypsin, 31 mg of chymotrypsin and 13 mg. Protease from S. griseus was used to replace the discontinued peptidase. The enzymes and casein were obtained from Sigma (Sigma Aldrich). The multi-enzyme solution was prepared fresh on the day of analysis and kept at 37 °C, the temperature at which its pH was adjusted to about 8.0 as described above. Upon rehydration, 1 mL of the multi-enzyme solution was added to the 10-mL sample dispersion, and the pH of the digesta was recorded every 5 second for 15 min. The change in pH at 10 min of digestion (DpH10 min) was used to calculate percent in vitro protein digestibility (IVPD) of the samples using following equation.

**In-vivo protein analysis:** The availability and digestibility of the protein in the quinoa protein isolates were determined by using the method of Matthews (2011) with some modification. Male Sprague Dawley rats were kept in the animal Room of the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad. The experiment was carried out by following the method of Moraes et al. (2012) with some modifications. According to that the experiment were carried on thirty male rats. The animals were randomly divided into six groups of five animals, so that the difference between mean weights did not exceed 2.2 g, as recommended by AOAC (2006). The rats were kept in individual stainless steel cages and maintained at 22±3°C with a 12 h light/dark cycle. The animal groups were fed with Protein-free diet, casein, and diets with quinoa protein. Ethical approval for the study was obtained from the ethics committee of National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad.

**Protein quality evaluation:** Net Feed intake and body weight gain were used to calculate growth study parameters including protein efficiency ratio (PER), net protein ratio (NPR). The protein efficiency ratio (PER) was determined by the method described by Moraes et al. (2012), which relates the weight gain of animals (g) with the protein intake (g). The diet, feces, urinary outputs, and dried rat bodies were analyzed for nitrogen content to work out nitrogen balance study parameter including true digestibility (TD), biological value (BV) and net protein utilization (NPU) as described by Moraes et al. (2012).

**Statistical analysis:** The data obtained for each parameter was subjected to analysis under complete randomized design (CRD) to determine the level of significance as described by Steel et al. (1997).

**Result**

**Chemical composition of quinoa:** It is revealed from the data that moisture content of quinoa genotypes varied significantly (Table 1). The highest value for moisture content was found in C. quinoa V2 (10.62±0.24%) and the lowest in C. quinoa V9 (9.74±0.22%). The highest ash content was found in C. quinoa V7 (2.80±0.02%) and the lowest in C. quinoa V2 (2.18±0.08%). The maximum value of crude protein was found in C. quinoa V7 which was 16.08±0.02% and minimum in C. quinoa V1 which was 11.00±0.34%. The highest crude fat was found in C. quinoa V2 (7.97±0.12%) and the lowest values was found in C. quinoa V9 (3.57±0.11%). The crude fiber ranged from 1.99±0.12 to 3.52±0.18% in the current study.

**Mineral composition of quinoa flour:** The mineral content of quinoa flour is shown in Table 2. It is obvious from the results that Calcium, Iron, potassium, magnesium, manganese, sodium, sulphur, Zinc, and copper ranged from 669.45±5.20 mg kg⁻¹ to 578.35±4.53mg kg⁻¹, 68.89±0.50 mg kg⁻¹ to 61.31±0.18 mg kg⁻¹, 9443.41±114.7mg kg⁻¹ to
Characterization of quinoa

<table>
<thead>
<tr>
<th>Quinoa genotypes</th>
<th>Moisture</th>
<th>Crude ash</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
</tr>
</thead>
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<td>3.52±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2.18±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.67±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.99±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
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Means in columns with similar letter are statistically non-significant (P>0.05)

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<th>C. quinoa V2</th>
<th>C. quinoa V1</th>
<th>C. quinoa V9</th>
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<td>621.13±6.37&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Copper</td>
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<td>5.04±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>4542.6±23.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4491.6±20.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4560.0±16.16&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1582.37±5.84&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Zinc</td>
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<td>29.40±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
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Means in rows with similar letter are statistically non-significant (P>0.05)

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<tr>
<th>Quinoa genotypes</th>
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<th>Oleic Acid</th>
<th>Linoleic Acid</th>
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<td>C. quinoa V7</td>
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<td>4.45±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>C. quinoa V1</td>
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<td>31.62±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5.38±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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Means in columns with similar letter are statistically non-significant (P>0.05)

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<tr>
<th>Quinoa genotypes</th>
<th>Water Absorption Capacity (%)</th>
<th>Oil Absorption Capacity (%)</th>
<th>Foaming Capacity (%)</th>
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<td>8.60±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>C. quinoa V1</td>
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<td>7.74±0.09&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>C. quinoa V9</td>
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<td>9.09±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
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Means in columns with similar letter are statistically non-significant (P>0.05)

8497.08±51.97 mg kg<sup>-1</sup>, 2173.40±23.12 mg kg<sup>-1</sup> to 2058.10±28.28 mg kg<sup>-1</sup>, 35.28±0.12 mg kg<sup>-1</sup> to 30.66±0.21 mg kg<sup>-1</sup>, 62.81±0.52 mg kg<sup>-1</sup> to 24.58±0.29 mg kg<sup>-1</sup>, 1606.30±8.29 mg kg<sup>-1</sup> to 1499.21±9.30 mg kg<sup>-1</sup>, 32.12±0.52 mg kg<sup>-1</sup> to 24.30±0.22 mg kg<sup>-1</sup> and 5.04±0.06 mg kg<sup>-1</sup> to 4.01±0.02 mg kg<sup>-1</sup>, respectively.

Fatty acid composition: Mean values regarding fatty acid composition have been illustrated in Table 3, that the highest value of palmitic acid was found in C. quinoa V9 and the lowest in C. quinoa V1 which were 13.25±0.30 and 11.39±0.02%, respectively. Other quinoa genotypes such as C. quinoa V2 and C. quinoa V7 possessed 12.06±0.06 and 11.76±0.18% palmitic acid, respectively. The highest value of oleic acid was found in C. quinoa V1 and the lowest in C. quinoa V2 which were 31.62±0.14 and 26.28±0.15%, respectively. In addition, the maximum value for linoleic acid was found in C. quinoa V7 and the minimum in C. quinoa V1 which were 52.84±0.28 and 47.79±0.19%, respectively whilst α-linolenic acid, the highest value was found in C. quinoa V7 and the lowest in C. quinoa V2 which were 7.71±0.06 and 4.45±0.03%, respectively.

Functional properties of quinoa protein isolates: It is obvious from the results (Table 4) that the quinoa protein isolates have highest value of water absorption capacity in C. quinoa V7 which was 3.82±0.05% and the lowest was C. quinoa V1 which was 2.81±0.17%. The mean values of oil absorption capacity depicted that C. quinoa V2 contained highest oil absorption capacity (3.03±0.03%) whereas C. quinoa V1 has lowest oil absorption capacity (2.72±0.02%). The variation with in different genotypes may be due to
difference in globular structure and particle size of protein. It is apparent from the results that the foaming capacity of *C. quinoa* V7, *C. quinoa* V2, *C. quinoa* V1 and *C. quinoa* V9 genotypes were 10.05±0.07, 8.60±0.01, 7.74±0.09 and 9.09±0.05%, respectively. The highest value of foaming capacity was found in *C. quinoa* V7 which was 10.05±0.07% and the lowest (9.09±0.05%) in *C. quinoa* V9.

**In-vitro digestion of quinoa protein:** The statistical data regarding in-vitro digestion of quinoa protein showed significant differences among quinoa genotypes (Table 5). The mean values for in-vitro protein digestibility has been presented in Table 5 which elucidated that quinoa genotypes have significant differences for the said parameter. The in-vitro protein digestibility of *C. quinoa* V7, *C. quinoa* V2, *C. quinoa* V1 and *C. quinoa* V9 were found to be 78.11±0.43, 76.74±0.57, 76.52±0.21 and 75.95±0.29%, respectively. The highest value of in-vitro protein digestibility was found in *C. quinoa* V7 which was 78.11±0.43% and the lowest in *C. quinoa* V9 (75.95±0.29%).

**Biological evaluation of quinoa protein:** Protein plays vital role in growth and development besides providing energy. It is obvious from results that the protein efficiency ratio differed significantly among different quinoa genotypes. The maximum protein efficiency ratio (3.78±0.05) among the experimental diets was found in *C. quinoa* V7 followed by diet comprised of *C. quinoa* V2 (3.76±0.04). The reference diets i.e. casein has PER value of 3.90±0.04. The mean values of NPR was observed that after reference casein diet (4.82±0.05), the highest value of net protein ratio (4.69±0.05) was exhibited in *C. quinoa* V7 while the lowest was observed in *C. quinoa* V1 (3.90±0.04).

Mean squares for true digestibility (TD), biological value (BV) and net protein utilization (NPU) demonstrated significant variations among the tested diets along with control diet. Among the tested diets maximum digestibility (90.57±1.10%) was observed in *C. quinoa* V9 protein isolates, followed by *C. quinoa* V2 (89.43±1.08%). However, diet containing casein showed true digestibility of 99.99±0.00%. Means for the effect of feeding diets containing quinoa on Sprague Dawley rats exhibited highest biological value of *C. quinoa* V9 (91.74±0.99%), followed by *C. quinoa* V2 (80.70±0.98%). However, rats fed on casein based diets exhibited 91.99±1.11% BV. The lowest NPU was observed in *C. quinoa* V1 i.e. 70.75±0.86%. However, the means for NPU were 94.18±1.14% for casein diet.

**DISCUSSION**

It is generally agreed that chemical composition of seed is primarily the most viable factor determining the nutritional composition and ilicit its food based importance. In this context, quite a number of physiological factors are believed to impart significant changes that involve variation in genetic makeup, agricultural practices and horticultural and physiological maturity of crop. Our findings are in harmony with the findings of Villa et al. (2014) who calculated protein content (11.7±0.2%) in quinoa. Moreover, they were quite of the opinion that environmental factors substantially play instrumental roles in having net protein contents. There has been a debate over the net protein contents of quinoa grains as variations do occur depending on the geographical region, soil condition and agronomic practice. In another attempt, it was proved that protein contents were 12.10±0.3% (Nascimento et al., 2014). The variation in protein content of seed might be due to soil fertility, application of fertilizers and availability of nitrogen to the plant. Likewise, in another instance involving moisture balance study in stored quinoa grains, Stikic et al. (2012) revealed that storage conditions have a viable role in assuring overall moisture level of grain. Additionally, external factors may lead to variations in the levels of said trait. It is also suggested that moisture content of grains is largely a matter dependent on cropping patterns, harvesting methods and storage conditions. Instant findings are in range with the previous work of Wright et al. (2002) who determined ash percentage in quinoa as ranged from 2.6 to 3.2%. In another finding, Repo-Carrasco-Valencia et al. (2010) reported crude ash contents in quinoa from 2.27±0.10 to 2.93±0.05%, however, variations in the ash percentage is mainly due to soil types, genetic variations, and the use of synthetic fertilizers (Miranda et al., 2009; Zielinski and Kozlowska, 2000).

Regarding mineral composition Konishi et al. (2004)
Characterization of quinoa

reported that quinoa have 40 mg kg\(^{-1}\) of zinc and 1500 to 2200 mg kg\(^{-1}\) of sulphur. Gonzalez Martin et al. (2014) found magnesium content in quinoa 1839.0±67.8 to 1912.1±213.0 mg kg\(^{-1}\). Hager et al. (2012) determined calcium content in quinoa (497.3±1.2mgkg\(^{-1}\)).The variation in mineral composition might be due to application of fertilization and soil fertility. The data acquired from present study regarding fatty acids have similar finding suggested by Ando et al. (2002) where it was reported that 10.3±0.2% of palmitic acid present in milled grain of quinoa. The results presented in this study are supported by the findings of Peiretti et al. (2013) where it was determined that quinoa has palmitic acid in the range of 114.7 to 129.4 g/kg at maturity stage (115.3 ± g/kg) was recorded. These results are in harmony with the findings of Miranda et al. (2012) who reported that different quinoa genotypes have oleic acids in the range of 18.68±0.27 to 27.87±0.02 g/100g. These results of present study are also confirmed by the findings of Calderelli et al. (2010) who explicated that quinoa have 48.8±0.41 % of linoleic acid. Rosero et al. (2013) explored that linoleic acid was most abundantly polyunsaturated fatty acid present in different varieties of quinoa which varied from 46.525 to 56.435 g/100g. Likewise, Valcarcel-Yamani and Lannes (2012) reported that α-linolenic acids ranged from 3.8 % to 8.3 % in quinoa. Rosero et al. (2013) investigated that quinoa has α-linolenic acid in the range of 6.721 to 8.814 g/100g. Wood et al. (1993) also reported that quinoa have 7.66±0.13 to 8.35±0.07% of α-linolenic acid among different varieties. Protein water absorption capacity is the characteristics of various factors like steric factor, conformational factors, size, shape along with balanced ratio of hydrophilic and hydrophobic sites of amino acids as well interaction of lipids, carbohydrate and tannins with protein. The findings of the current research is similar to the results of the Chauhan et al. (1999) who found that the water holding capacity of quinoa protein isolate was 5.4±0.2 g/g on protein weight basis. The water absorption capacity measured mainly by the content and the level of hydration of the insoluble fraction of a protein isolate. On the other hand, protein isolates showed superior solubility revealed lower water holding because these contain a low proportion of insoluble protein fraction. The variations in water absorption capacity of protein isolates might be due to conformational characteristics and protein concentration (Chavan et al., 2001). Oil absorption capacity of protein isolates showed the occurrence of hydrophilic and hydrophobic groups on the surface of the protein molecules which effect the oil absorption capacity of protein isolates. The present results are in relation with the findings of Chandi and Sogi (2007) who elucidated that the oil absorption capacity of casein and rice protein isolated was 1.72 ± 0.09 % and 6.74 ± 0.34%, respectively. Foaming capacity of protein isolates varied might be due to insufficient electrostatic repulsions, and interactions of protein and protein to form protein aggregates which are unfavorable for formation of foam. The results are in line to the findings by Aluko and Monu (2003), who determined that quinoa protein comprised of a very low foaming capacity, which was due to the globular nature of the protein. The globular nature reduced its ability to form interfacial membranes around air bubbles. It has been reported that variations in foaming capacity of protein isolates might be due to rapid unfolding at the air and water interface, increased in solubility, restricted intermolecular cohesion along with flexibility of the protein surfactant molecules (Chavan et al., 2001). In-vitro digestion of protein effected by occurrence of starch and protein interaction as well as depended on molecular weight of protein. The findings of the current study are in line with the previous study of Repo-Carrasco-Valencia and Serna (2011) who observed that the in-vitro protein digestibility of quinoa varied between 76.3-80.5% among different varieties. Protein efficiency (PER) results of current study are supported by the research of Mahoney et al. (1975) who explicated that raw quinoa and cooked quinoa have PER about 2.09 and 2.71, respectively. Protein from varied origins and their proportions in the formulations can result in variations in the amino acid concentrations, which ultimately alter its efficiency when consumed by individuals (Silva et al., 2014). The findings of current research supported by Dijkstra et al. (2003) who reported that quinoa protein have 84% true digestibility. It was reported that quinoa have 43-51% biological value Dijkstra et al. (2003). Means for the effect of quinoa protein isolates on Sprague Dawley rats showed that C. quinoa V9 have better net protein utilization (73.78±0.89%). The results of current findings are in line with the findings of Guzman-Maldonado and Paredes-Lopez (1998) who reported that quinoa have 76% NPU. It has been earlier reported that some nutritional and anti-nutritional factors like tannin, phytic acid and dietary fiber can also effect at some extent in digestion of protein (Ghavidel and Prakash, 2007).

Conclusion: The use of quinoa is of great nutritional interest owing to its composition. Quinoa has higher nutritional profile i.e. fatty acids, and minerals as compared to other cereals. In addition it is good source of protein. This composition and nutritional facts describes their potential for functional properties and human health. In the nutshell, quinoa holds potential to be utilized in cereal base products for best quality and value addition because of its functional properties.

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Characterization of quinoa


