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A comparative study of protein and free amino acid contents in some important ancient wheat lines

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RESEARCH ARTICLE

Abstract

In this study, protein content and amino acid compositions of some selected ancient wheat lines (forty-nine emmer wheat (*Triticum turgidum dicoccum*) and thirty-six einkorn (*Triticum monococcum* L.) were determined to evaluate the nutritional quality of these two wheat species. The results were compared with three control cultivars of durum wheat (*Triticum turgidum durum*). Protein content was measured by a Dumas combustion method and amino acid profiles (threonine, lysine, arginine, histidine, aspartic acid, cysteine, glutamic acid, serine, proline, valine, methionine, tyrosine, leucine+isoleucine and phenylalanine) were analysed by a liquid chromatography tandem mass spectrometry. Protein contents of einkorn, emmer wheat lines and durum wheat cultivars were found to be 21.29±1.59, 17.35±2.49 and 16.93±1.76%, respectively. The essential amino acids and protein contents determined in the analysis were higher in einkorn lines than those of emmer wheat lines and control cultivars. In conclusion, the grain of einkorn lines can be selected as the most suitable raw material for human nutrition due to the quality of their protein and amino acid contents.

Keywords: amino acid, ancient wheat, einkorn, emmer wheat, protein

1. Introduction

Wheat, the principal source of energy and protein for majority of the world's population, is one of the most important cereal crops. Ancient wheat species, like emmer and einkorn wheat, have been recently receiving increased attention due to their high levels of protein, dietary fibre, minerals and phytochemicals (phenolic acids, alkylresorcinols, tocols, carotenoids and B vitamins) (Gebruers *et al.*, 2008; Hejtmánková *et al.*, 2010; Hidalgo and Brandolini, 2008; Lachman *et al.*, 2013; Shumoy and Raes, 2017; Zhao *et al.*, 2009). These species have also been recommended for inclusion in diet of patients treated for health problems, like high blood cholesterol, allergies, colitis, rheumatoid arthritis, depression and cancer (Strehlow *et al.*, 1991, 1994). Therefore, it can be presumed that the grains of ancient wheat species are suitable to produce functional food products and to meet increasing demand for more nutritious food commodities. Shaheen *et al.* (2016) reported that the mean concentration of protein in wheat grain and flour was 10.6 and 12.1%, respectively. They also determined that the amino acid (phenylalanine, tyrosine, histidine, isoleucine, leucine, lysine, cysteine, methionine, threonine, tryptophan, valine, arginine, alanine, aspartic acid, glutamic acid, glycine, proline and serine) contents were 475, 270, 229, 307, 691, 276, 218, 220, 300, 124, 450, 440, 556, 505, 3,537, 412, 1,065 and 505 mg/100 g, respectively.

The well-known and widely studied ancient wheat species are the diploid wheat einkorn (*Triticum monococcum* var. *monococcum* with AA genome), tetraploid emmer (*Tiriticum dicoccum* Schrank ex Schübl. with AABB genome) and hexaploid spelt (*Tiriticum aestivum* var. *spelta* with AABBDD genome) (Shewry and Hey, 2015). Einkorn is a primitive wheat of interest due to its high level of protein, carotenoids and phosphorus (Abdel-Aal and Hucl, 2002). Breads produced from einkorn lines were comparable to those of baked from high quality wheat. Borghi *et al.* (1996) evaluated 25 einkorn lines in bread making and

demonstrated their valuable content of carotenoids and proteins for the production of bakery products (Borghi *et al.*, 1996). Furthermore, it was reported that einkorn has high polyphenol oxidase activity and relatively low bound polyphenol contents (Hidalgo and Brandolini, 2014). Emmer wheat is one of the ancestors of modern durum wheat genotypes. Konvalina *et al.* (2009) analysed amino acid composition of 6 emmer wheat varieties from different genetic background and reported that the grains of emmer wheat can be incorporated into a nutritionally complete diet (Konvalina *et al.*, 2009). Spelt, another ancient wheat species, may contain higher levels of protein, soluble dietary fibre and minerals than those of modern bread wheat genotypes. The tocopherols, minerals, protein, starch, and dietary fibre compositions of spelt have also been investigated and compared with common wheat varieties (Hussain *et al.*, 2012; Ranhotra *et al.*, 1996; Zhao *et al.*, 2009).

Although the ancient wheat species were widely cultivated in the past, they are currently cultivated in limited areas of Europe, Asia and Australia. One reason why the cultivation of ancient wheat species has declined is that common wheat species are usually bred for high input intensive systems while ancient wheat species are usually grown in organic and traditionally low input farming systems (Shewry and Hey, 2015). In particular, the cultivation of einkorn and emmer wheat has significantly decreased with the widespread use of high-yield durum (*Triticum durum* L.) and bread wheat (*T. aestivum* L.) cultivars Kaplan *et al.* (2014). However, einkorn and emmer wheat are still cultivated in the marginal areas of the Mediterranean region, especially in Turkey and the Balkan countries (Brandolini and Hidalgo, 2011). Turkey is considered as the genetic diversity centre of wild relatives of wheat because the country hosts 14 different species of wheat compared to 22 species in Middle East, Mediterranean, and West Asian gene centres (Van Slageren, 1994). Therefore, the einkorn and emmer wheat landraces of Turkey can globally provide genetic resources for wheat breeding programs.

Ancient wheat species differ from common bread and durum wheat species due to their hull characteristics (i.e. the glumes remaining tightly closed over the grain and not removed by threshing). Compared to the common bread and durum wheat, hulled wheat can easily be cultivated under adverse climate and soil conditions (Hammer and Perrino, 1984; Kaplan *et al.*, 2014; Mielke and Rodemann, 2007). Hulled wheat also has strong tillering ability, resistance to drought and fungi, competitiveness to weeds, and higher protein and amino acid contents (Løje *et al.*, 2003; Moudry, 1999; Rüegger *et al.*, 1990; Schmid *et al.*, 1994). From the nutritional point of view, wheat proteins are low in some amino acids essential for the human diet, especially lysine (the most deficient amino acid) and threonine (the second limiting amino acid) but they are also rich in glutamine and proline (Abdel-Aal and Hucl, 2002). Lysine content of cereal crops was reported to be 1.80-2.00, 2.50-3.20, 3.50-4.00, 2.90-3.20, 3.80-4.00 and 2.00-2.80 for maize, wheat, rice, barley, oats and sorghum, respectively (Vasal, 2004). The quality of wheat also depends on the nutritional value of its protein fraction and a balanced essential amino acid contents in the protein complex is important (Hussain *et al.*, 2012). For that reasons, ancient wheat species can be good alternatives for balancing the human nutrition due to their valuable nutrition content and resistance to harsh environmental conditions. There are significant number of studies on the composition of bioactive components and functional properties in ancient wheat species (Bonafaccia *et al.*, 2000; Giambanelli *et al.*, 2013; Løje *et al.*, 2003; Serpen *et al.*, 2008), however few studies have been done for comparison of amino acid profiles of these ancient species with modern wheat varieties.

Therefore, the main objectives of the present study were to compare selected ancient wheat lines of einkorn and emmer wheat to modern wheat varieties for a range of essential and non-essential amino acids and protein contents and to evaluate their lines for higher protein and amino acid values for human health and food quality.

2. Materials and methods

Wheat samples and experimental design

Forty-nine emmer wheat and 36 einkorn lines used in the field experiments were collected from different wheat cultivated areas of Turkey such as Sinop, Kastomonu and Kayseri provinces. These lines were selected from 120 different populations by using single spike selection over the last five years. Einkorn and emmer wheat lines were separately planted in two different experiments with an augmented experimental design proposed by Kling and Merk (2012) in 2015-16 growing seasons at the Agricultural Faculty Experimental Farm of Akdeniz University in Antalya, Turkey.

Commonly cultivated durum wheat varieties, Sarıçanak, Svevo and Zenith, were also used as control cultivars for the comparison of protein and amino acid contents of the ancient wheat samples. The control cultivars have been widely grown and used as check cultivars under national registration trials in Turkey. Moreover, einkorn in general and emmer wheat in particular have been classified as durum wheat groups based on their consumption such as pasta and bulgur (Anonymous, 2017).

The control samples were planted in both the einkorn and emmer wheat experiments, where the all three controls were replicated in each plot while each line was unreplicated in the experiments. Einkorn and emmer wheat experiments were consisted of six blocks in order to get a minimum of

10 degrees of freedom. There were a total of 54 plots in the einkorn experiment including 36 lines and 3 controls. Therefore, each block was consisted of 6 different einkorn lines and 3 replicated control cultivars. On the other hand, there were a total of 67 plots in the emmer wheat experiment including 49 lines and 3 controls, so that each block consisted of 12 different emmer wheat lines and 3 replicated control cultivars. Additionally, the sixth block of the trial had only 7 emmer wheat lines and three durum wheat checks. The experimental design and statistical models were employed as suggested by Kling and Merk (2012). The statistical model reported was given below:

$$Y_{ij} = \mu + \beta_i + C_j + \tau_{k(i)} + \varepsilon_{ij}$$

Y_{ij} = Mean + Blocks + Checks + New entries + Error

Average temperature was lowest in January (3 °C) and highest in June (35 °C) and total seasonal precipitation was 450 mm. Average temperature value in the growing season was 2 °C higher than long term average and there was 200 mm less rainfall than the average. In order to reduce the negative effect of severe drought during the cultivation period, the experimental plots were irrigated twice at the mid of stem elongation (Z32-Z39) and just beginning of heading (Z55) times. The soil texture of the experimental area had slightly alkaline, silty clay, extremely calcareous, reasonable phosphorus and potassium with poor organic matters. The characteristics of the soil were suitable for growing wheat (FAO, 1990).

Einkorn and emmer wheat lines along with 3 controls were planted in the second week of December of 2015 at a sowing rate of 200 seeds/m^2 (Troccoli and Codianni, 2005). 80 kg/ha N and 80 kg/ha P_2O_5 were applied to the soil during planting time. Each plot had 4 rows that were 3 m in length and 14 cm in width and emmer wheat and einkorn lines were manually harvested in the second week of June in 2016. The samples harvested were de-hulled and ground by a laboratory mill prior to analysis. The particle sizes of the wheat samples were 75 µm and the moisture of the samples was 11.5%.

Reagents and standards

The stock amino acid mixtures (L-arginine (Arg), L-aspartic acid (Asp), L-cystine (Cys), L-glutamic acid (Glu), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tyrosine (Tyr), L-valine (Val)) used in this experiment were obtained from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (98%), ammonium formate (99%) and HPLC grade methanol (99.8%) were also obtained from Merck (Kenilworth, NJ, USA). Ultrapure water was produced by a Millipore ultrapure water (18.2 MΩ cm at 25 °C) purification system (Bedford, MA, USA).

The amino acids in the stock, were at 2.50 µM/ml in 0.1 M HCl. Calibration standards were prepared from the stock by making different fold dilutions in acidified methanol: water (95:5) and stored in amber glass at -20 °C.

The calibration range depended on each amino acid's molecular weight so that it changed between 0-1.88 mg/kg and 0-15 mg/kg for the lowest molecular weight amino acid, Ser, and the highest molecular weight amino acid, Cys, respectively.

Protein analysis

The total protein contents of the wheat samples were determined through pyrolysis by measuring total nitrogen content using Velp NDA/701 protein analyser (Velp Scientifica, Usmate, Italy) based on a modified Dumas method (Cunniff, 1995). According to the method, one hundred milligrams of the wheat sample were combusted in a sealed furnace combined with a thermal conductivity detector (Velp Scientifica). The total protein percentage was calculated by measuring the total nitrogen content in the sample assuming a wheat protein to nitrogen conversion factor of 5.33 (Chang, 2010).

Amino acid analysis

A 0.5 g of each sample was ground into powder in 15 ml PP centrifuge tube. Ten ml of acidified water were used as extraction solvent (80:20 water: methanol, 0.1% formic acid). The solvent was added into the tube and vortexed for 5 min. The tube was centrifuged at 1,792 *rcf* for 15 min at 4 °C in a centrifuge (Eppendorf, Hamburg, Germany). The supernatant was filtered through a 0.2 µm PTFE syringe filter. The filtrate was diluted with mobile phase A and injected into a liquid chromatography tandem mass spectrometer (LC-MS/MS) (Kivrak *et al.*, 2014). The method was optimised for preparation of the calibration curves and calculation of the limits of detection and the quantification (LOD and LOQ).

Instrumentation and chromatographic conditions

The instrumental analysis was performed with a LC-MS/MS system (Thermo Scientific, San Jose, CA, USA). The instrument was composed of a Thermo Accela High Performance Liquid Chromatography (LC) and TSQ Access Max Tandem Mass Spectrometer. The electrospray ionization (ESI) technique was used for ionization of the compounds. Reverse-phase chromatography compatible with Thermo Hypersil Gold 100×2 mm ID, 1.9 µm particle sized column was also included in the analysis. The mobile phase was consisted of aqueous 0.1% (v/v) formic acid and

4 mM ammonium acetate in 5% methanol (mobile phase A) and 100% methanol (mobile phase B). The gradient flow was started with 100% mobile phase A and held at 100% for 1.5 min. Aqueous phase percentage was decreased to 0% in 2 minutes and held at 0% for 3 min. At the final phase, the flow was adjusted to 100% mobile phase A to turn back to the initial condition. Total injection duration, injection volume and flow rate were 7 min, 10 µl and 0.4 ml/min, respectively.

The mass spectrometer was adjusted to the following parameters: capillary temperature was set at 320 °C, ESI electrospray voltage at +3,200 V, sheath gas pressure at 30 l/min, aux gas pressure at 10 l/min. In the collision cell, gas pressure was set at 1.5 m Torr. For detection of amino acids, two MS/MS transitions were used: one for quantification and the other one for peak confirmation. Data analyses were carried out with Xcalibur software (Thermo Finnigan, Waltham, MA, USA).

The LC-MS/MS method used in the study showed a satisfactory performance for the quantification of the tested amino acids in the wheat samples. High linearity and correlation coefficient values (>0.998) were achieved for the amino acids analysed under the chromatographic conditions. The LOQ was estimated by standard deviation derived from ten different analyses of the sample spiked with the lowest calibrated level (LCL). The LOQ levels were found to be 0.87, 0.67, 1.20, 0.74, 0.78, 0.66, 0.66, 0.73, 0.75 , 0.83, 0.58, 0.53, 0.60, 0.91 and 0.59 mg/kg for arginine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine, respectively. The remaining five amino acids could not be determined in the system due to the formic acid interference in the mobile phase.

Statistical analysis

Data on protein and amino acid profiles of emmer, einkorn and durum wheat cultivars were statistically analysed by using Minitab 16 software (Minitab Ltd, Coventry, UK). Means and standard errors of the genotypes were calculated including large number of genotypes with small quantity of seeds. Correlation analysis was performed for all the data to show the relationship between protein contents and different amino acid profiles. The total variations in all traits investigated in these two experiments were determined by using principal component analysis (PCA). Finally, all protein and different amino acids data were bi-plotted to demonstrate the relationships among traits in all einkorn, emmer wheat lines and durum wheat cultivars in the same chart.

3. Results and discussion

Protein content of the samples

Grain protein content of the samples varied from 14.91 to 18.15% for durum wheat, 13.51 to 23.92% for emmer wheat and 15.76 to 24.75% for einkorn. The einkorn lines had the highest mean protein content, followed by emmer wheat lines whereas the durum wheat lines had the lowest concentration of protein. Einkorn lines of 32 and 4 were relatively higher mean protein contents than those of durum and emmer wheat. The high protein content of einkorn lines can be attributed to their small 1000 kernel weights as pointed out by Gurcan *et al.* (2017). There was a significant difference in protein content between emmer wheat and einkorn lines ($P<0.05$). However, no significant difference was found between protein content of emmer wheat and durum wheat ($P<0.05$). Protein content of the einkorn samples in the present study was quite similar to that of previously reported Italian einkorn wheat (Hidalgo *et al.*, 2016). In addition, einkorn line 32 had the highest concentration of protein and therefore it could be selected for protein enrichment of wheat products.

Amino acid profile of the samples

The LC-MS/MS analysis of the emmer wheat, einkorn and durum wheat samples revealed the amino acid concentrations of 15 amino acids including seven essential and eight non-essential amino acids (Table 1). Variations in amino acid contents among the lines were presented in Figure 1 and 2. The means of the analysed essential amino acids were determined to be higher in einkorn lines than those of emmer wheat lines and control cultivars. In addition, the highest concentrations of non-essential amino acid except arginine and proline were found in the einkorn samples. These amino acids were determined to be highest in the emmer wheat samples. Amino acids in einkorn were exceptionally high in terms of leucine+isoleucine, phenylalanine, glutamic acid, lysine, tyrosine and aspartic acid. A similar finding was reported by Abdel-Aal and Hucl (2002) which found that glutamic acid and aspartic acid had the highest proportion in whole amino acid contents in selected ancient wheat species (einkorn, hard spelt, soft spelt, khorasan, durum and hard red spring wheat). In contrast, the contents of lysine, leucine+isoleucine, aspartic acid and proline were found to be different from the study of Shaheen *et al.* (2016). This difference can be a consequence of amino acid determination method used in the analyses. In this study, free amino acid method was used, while a digestion procedure was used in the study by Shaheen *et al.* (2016). Additionally, when compared to emmer wheat and durum wheat, einkorn line 6 had an extremely high concentration of threonine, lysine and phenylalanine. Similarly, this study revealed that einkorn

Table 1. Amino acid profiles (mg/kg) and protein contents (%) of the samples.

Par.	Type	Emmer wheat (n=49)			Einkorn (n=36)			Durum wheat (n=3)		
		Mean±SD	Medium	Range	Mean±SD	Medium	Range	Mean±SD	Medium	Range
PRT		17.38±2.54	17.01	13.51↔23.92	21.29±1.59	21.25	15.76↔24.75	16.93±1.76	17.73	14.91↔18.15
Thr	Essential	28.93±11.02	29.44	8.70↔53.16	41.67±11.95	41.50	18.06↔76.31	20.89±5.10	20.18	16.18↔26.31
Lys	Essential	395.94±132.63	368.92	149.72↔767.16	1,320.72±359.52	1,303.89	689.96↔2,313.04	204.61±122.43	266.62	63.58↔283.62
Val	Essential	29.01±67.03	0.00	0.00↔318.39	90.09±142.29	0.00	0.00↔548.06	0.00±0.00	0.00	0.00↔0.00
Leu+Ile	Essential	34.09±8.54	33.55	18.64↔57.49	102.25±20.55	98.11	78.01↔165.71	26.18±5.73	25.86	20.61↔32.06
Phe	Essential	51.68±6.76	52.96	38.98↔68.87	88.56±10.45	88.47	66.65↔110.98	39.98±7.07	42.24	32.06↔45.65
Met	Essential	12.23±5.53	11.66	3.85↔26.87	21.13±6.44	20.18	10.29↔40.64	3.65±2.47	4.27	0.93↔5.75
Arg	Non-essential	23.94±12.84	22.45	0.51↔52.95	20.99±11.31	21.54	0.97↔44.54	37.54±8.74	39.59	27.96↔45.08
His	Non-essential	14.83±3.53	14.33	9.50↔28.97	27.42±5.19	27.06	17.93↔40.31	12.63±1.36	12.90	11.16↔13.83
Glu	Non-essential	416.09±78.91	409.63	263.03↔661.04	695.60±91.87	694.34	507.41↔879.17	217.85±70.27	245.03	138.05↔270.46
Asp	Non-essential	374.59±51.72	380.07	242.24↔491.97	1,139.29±143.57	1,158.97	643.26↔1,503.80	253.91±78.57	275.85	166.70↔319.19
Cys	Non-essential	4.05±5.25	0.00	0.00↔20.00	10.65±12.49	8.59	0.00↔51.19	12.08±15.67	6.46	0.00↔29.79
Ser	Non-essential	45.06±84.46	0.00	0.00↔332.63	66.49±161.16	0.00	0.00↔654.20	75.43±130.65	0.00	0.00↔226.29
Pro	Non-essential	37.27±41.07	29.15	12.89↔308.40	94.41±32.47	91.86	42.99↔184.46	28.35±3.61	29.13	24.41↔31.51
Tyr	Non-essential	40.73±12.06	37.82	22.30↔77.11	92.48±12.85	91.85	63.43↔121.80	36.91±18.11	44.07	16.31↔50.33

lines 37, 56 and 26 contained the highest levels of valine, leucine+isoleucine and methionine, respectively (Figure 1).

The content of 15 amino acids ranged between >LOQ and 2,313 mg/kg. The highest concentration of lysine was 2,313 mg/kg, followed by aspartic acid 1,504 mg/kg, glutamic acid 879.2 mg/kg and serine 654.2 mg/kg. The high content of lysine in all wheat samples studied can be attributed to the analytical technique which is employed first time for the determination of amino acids using a non-digested sample preparation step. This may be explained by the low solubility of the lysine in the digestion solutions. It can be noticed that lysine, aspartic acid and glutamic acid were the dominant groups for all analysed wheat samples (Figure 3). This result is in agreement with the results of another study that reported glutamic acid was the major amino acid in wheat samples (Hussain *et al.*, 2012).

Linear correlations among the analysed parameters were presented in Table 2 and 3. There were significant positive relationships (≥0.700) between lysine and histidine; lysine and glutamic acid; lysine and methionine; glutamic acid and methionine; lysine and tyrosine; histidine and tyrosine; glutamic acid and tyrosine; methionine and tyrosine, lysine and leucine+isoleucine; methionine and leucine+isoleucine; tyrosine and leucine+isoleucine; leucine+isoleucine and phenylalanine parameters in emmer wheat samples. Significant positive relationships were also observed in einkorn samples between lysine and histidine; lysine and glutamic acid; histidine and glutamic acid; proline and methionine; proline and leucine+isoleucine; histidine and phenylalanine; tyrosine and phenylalanine parameters. The proportion of lysine was positively influenced by protein content in all the samples (P<0.05) as indicated by Shoup *et al.* (1966).

Score and loading plot based on chemometric evaluation of emmer wheat and einkorn samples showed that PC1 and PC2 accounted for 60 and 9% of the variation, respectively (Figure 4). Totally, 69% of cumulative variation was explained by these two main components. This clearly pointed out that einkorn lines had the best balance of essential amino acids compared to the lines of other species. According to score plot for protein and amino acid contents, there was generally polarization of einkorn from emmer genotypes as two distinct groups. The result demonstrated that PCA is a powerful tool for the categorization of einkorn and emmer wheat lines into two distinct groups based on protein and amino acid contents.

Total essential amino acid contents (threonine + lysine + valine + methionine + leucine + isoleucine + phenylalanine), were determined to be 1,686, 596.3 and 312.2 mg/kg for einkorn and emmer wheat and durum wheat, respectively in the study. Significant differences were observed in the total essential amino acid content among the wheat species

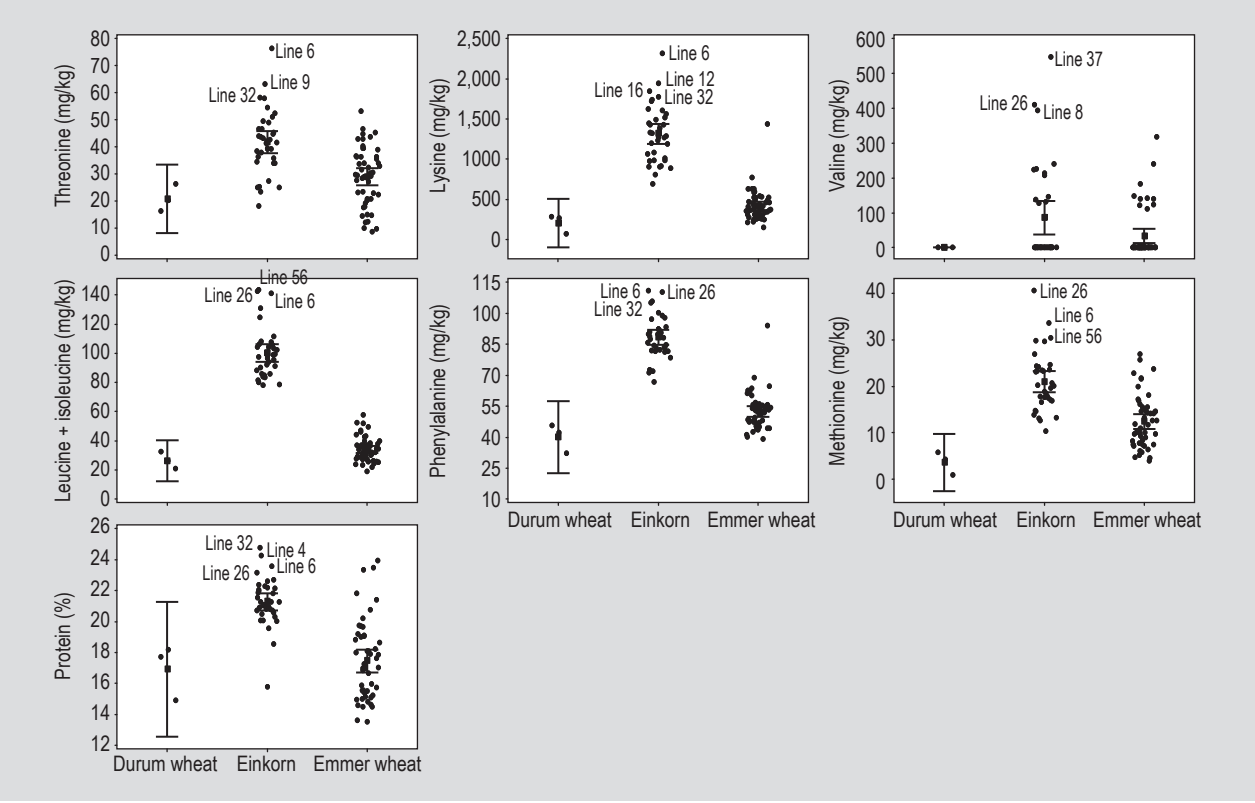

Figure 1. Individual value plots for essential amino acids and protein content of durum wheat, einkorn and emmer wheat with confidence intervals (95%).

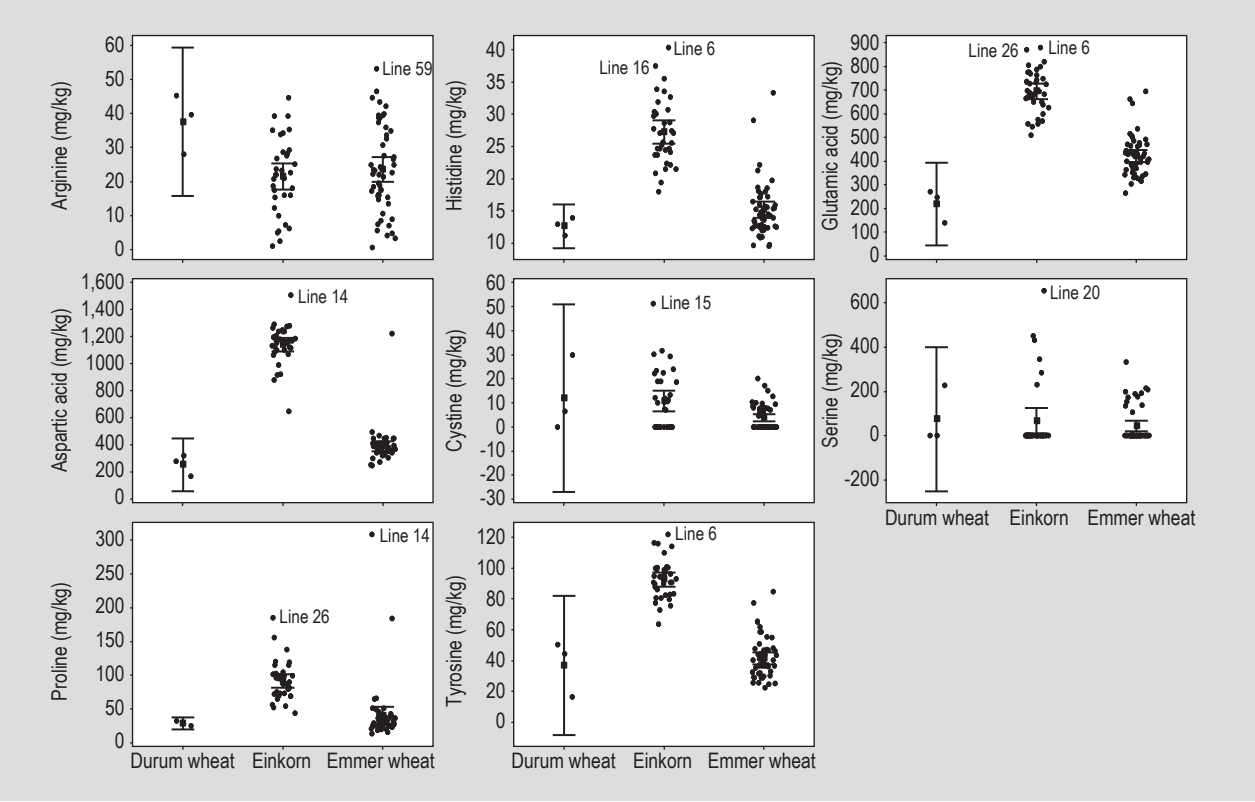

Figure 2. Individual value plots for non-essential amino acids of durum wheat, einkorn and emmer wheat with confidence intervals (95%).

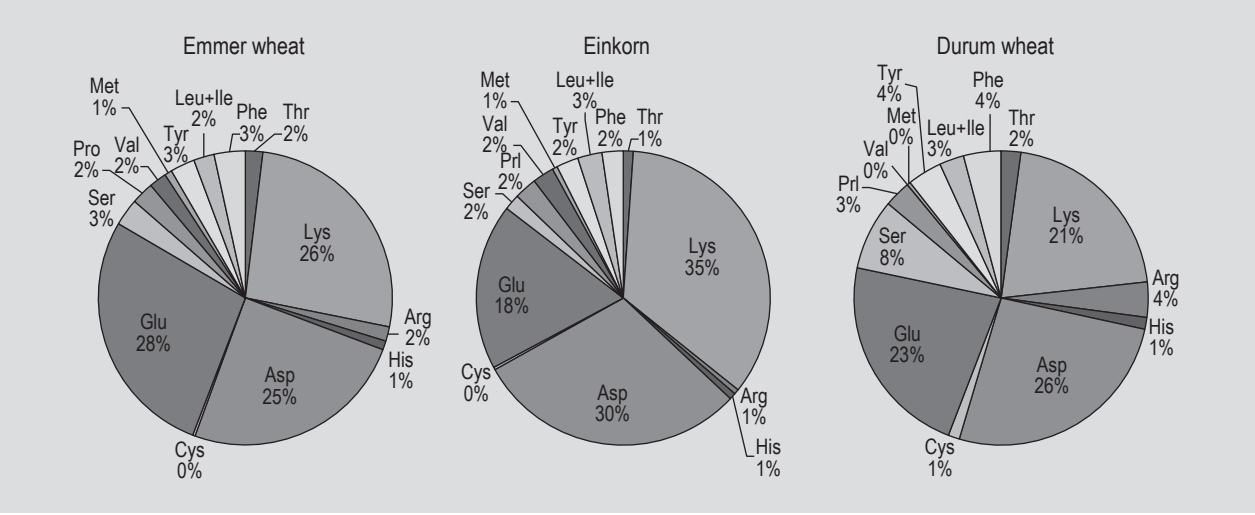

Figure 3. Percent amino acid variations for emmer wheat, einkorn and durum wheat.

Table 2. Relationship among quantitative parameters of emmer wheat.

	PRT	Thr	Lys	Arg	His	Asp	Cys	Glu	Ser	Pro	Val	Met	Tyr	Leu+Ile	Phe
PRT	1.00														
Thr	0.30	1.00													
Lys	0.61	0.44	1.00												
Arg	0.36	0.25	0.60	1.00											
His	0.43	0.33	**0.70**	0.57	1.00										
Asp	0.07	0.02	0.07	0.04	-0.01	1.00									
Cys	-0.07	0.12	0.09	0.08	0.11	-0.17	1.00								
Glu	0.45	0.52	**0.83**	0.57	0.67	0.21	0.18	1.00							
Ser	-0.26	-0.13	-0.31	-0.23	-0.27	0.11	-0.03	-0.31	1.00						
Pro	0.15	0.23	0.10	0.08	0.24	0.33	0.17	0.23	0.21	1.00					
Val	0.20	0.22	0.26	0.04	0.26	-0.13	-0.17	0.26	-0.14	0.01	1.00				
Met	0.47	0.35	**0.79**	0.55	0.63	-0.06	0.07	**0.72**	-0.39	0.03	0.36	1.00			
Tyr	0.44	0.43	**0.75**	0.64	**0.80**	-0.06	-0.03	**0.74**	-0.34	0.18	0.42	**0.80**	1.00		
Leu+Ile	0.30	0.38	**0.71**	0.50	0.64	0.14	0.06	0.68	-0.19	0.34	0.40	**0.74**	**0.70**	1.00	
Phe	0.33	0.32	0.58	0.38	0.68	0.29	0.15	0.67	-0.14	0.39	0.26	0.61	0.68	**0.74**	1.00

(P<0.05). Additionally, the percentages of essential amino acids in the samples were 43.98, 38.40 and 31.64% for einkorn, emmer wheat and durum wheat, respectively. The findings indicated that the einkorn was the richest sources of essential amino acids compared to emmer wheat and durum wheat species.

4. Conclusions

Ancient wheat species (einkorn and emmer wheat) are potential food sources with good nutritional properties due to their valuable protein and amino acid contents. The protein and essential amino acid contents were determined to be highest in einkorn lines. in comparison to emmer and durum wheat lines. Additionally, non-essential amino acid compositions except arginine and proline were also higher in einkorn than the other analysed wheat species. In conclusion, the grains of einkorn can be used for the production of nutritional supplements and alternative wheat products. Moreover, some selected lines (line 4 for protein content, line 6, 26 and 32 for amino acid contents) of einkorn can be used as parents to improve grain protein and essential amino acid content in wheat breeding programs. They can also be used for the enrichment of the wheat flour and semolina which contain low levels of essential amino acids. In order to verify these data, advanced lines with high protein contents should be tested in different locations and years.

Table 3. Relationship among quantitative parameters of einkorn.

	PRT	Thr	Lys	Arg	His	Asp	Cys	Glu	Ser	Pro	Val	Met	Tyr	Leu+Ile	Phe
PRT	1.00														
Thr	0.17	1.00													
Lys	0.55	0.30	1.00												
Arg	0.21	0.07	0.52	1.00											
His	0.46	0.16	**0.88**	0.40	1.00										
Asp	-0.04	0.00	0.27	-0.05	0.34	1.00									
Cys	0.07	-0.15	0.00	0.02	-0.01	0.07	1.00								
Glu	0.32	0.13	**0.73**	0.33	**0.73**	0.60	0.18	1.00							
Ser	0.09	0.26	0.02	0.00	-0.24	-0.11	-0.02	-0.07	1.00						
Pro	0.34	0.00	0.54	0.23	0.58	0.10	0.03	0.39	-0.23	1.00					
Val	0.07	-0.18	0.15	0.06	0.16	0.12	0.31	0.19	-0.21	0.37	1.00				
Met	0.50	0.11	**0.70**	0.49	0.62	0.20	0.20	0.62	-0.06	**0.78**	0.31	1.00			
Tyr	0.32	0.26	0.65	0.32	0.61	0.54	0.03	**0.77**	-0.08	0.22	-0.04	0.45	1.00		
Leu+Ile	0.32	-0.02	0.53	0.16	0.57	0.28	-0.09	0.43	-0.21	**0.86**	0.26	0.65	0.34	1.00	
Phe	0.39	0.22	**0.75**	0.37	**0.72**	0.50	0.06	**0.77**	-0.11	0.53	0.12	0.64	**0.82**	0.67	1.00

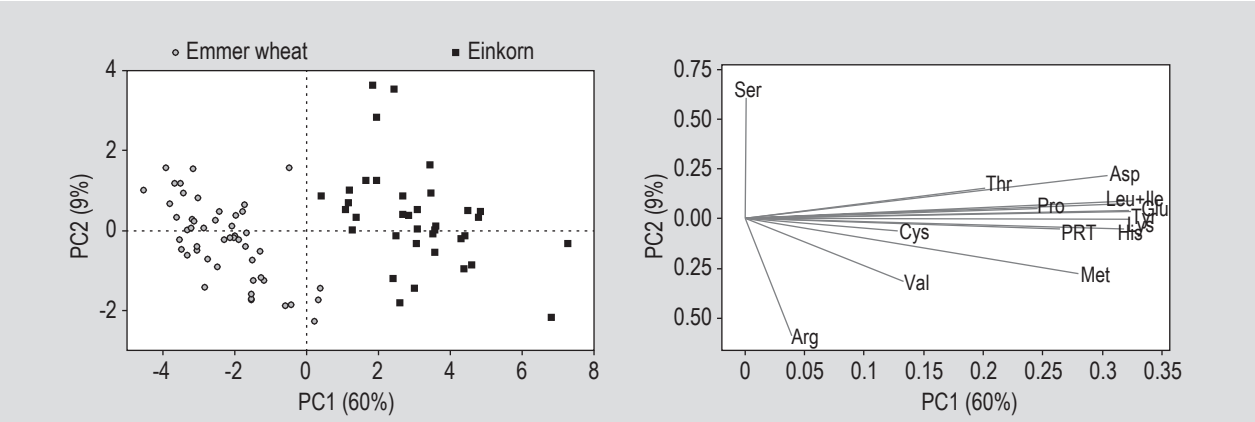

Figure 4. Score and loading plot of emmer wheat and einkorn samples based on 2 main principal components.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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