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Antioxidant Activity and Essential Amino acid Content of Bread Wheat (*Triticum aestivum* L.) Varieties

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ABSTRACT

In recent years, consumption of healthy and nutritious products has come to the forefront with the increased consumers' awareness because of sensitivity to human health. Bread wheat provides the important proportion of protein, fiber, mineral, and antioxidant compounds that detected wheat and wheat-based products in daily consumption. The study aimed to provide new insights and results on the antioxidant properties and essential amino acid profile of 45 bread wheat varieties collected from different ecological regions of Turkey. Antioxidant activity was measured using DPPH radical and total phenolic content was determined by using the Folin-Ciocalteu method used gallic acid as standard. Essential amino acid content was evaluated by oxidation and hydrolysis procedure by HPLC analysis. As a result of the study, significant differences and correlations were found between the varieties and all evaluated parameters. Total phenolic content and antioxidant activity values respectively ranged between 102.4 and 211.8 µg GAE/g (gallic acid equivalent/g), 11.8 and 26.3% inhibition in whole wheat flour. Amino acid amount and profile were significantly affected by variety and the most limiting amino acid lysine showed a wide range (0.39-1.47 g/100 g flour) and the concentration of leucine and phenylalanine were found to be higher in wheat compared to the other essential amino acids. Correlation analysis between protein and essential amino acids demonstrated a positive correlation with high values. Clustering analysis highlighted an important genetic diversity, suggesting that winter growth habit varieties have valuable nourishment properties with high protein and amino acid composition. These data suggest that health and nourishment properties influenced differently by genotype potential of wheat grain.

Keywords: Whole wheat, Antioxidants, Protein quality, Lysine, DPPH, HPLC, Cluster

1. Introduction

Wheat is a major crop of cereals grown across the world because widely adapted to most climate and soil conditions. Bread wheat is an important food for daily consumption to provide energy and essential nutrients due to its high content of carbohydrate and protein content. In addition to basic nutrients, wheat is also a good source of phytochemicals which can provide additional health benefits (Narwal et al. 2012). Whole grain products are recommended for healthy diets as a source of dietary fiber and antioxidant substances. Biotic and abiotic stress factors lead to the formation of free radicals in plants and our bodies that damage body cells, cell walls or even DNA. Some chemicals (phenolic acids and vitamins) which are taken from the nutrients show antioxidant activity in the human body, prevent these radicals from causing damage. It is known that cereals (especially whole grain products) also have antioxidant activity potential compared to fruits and vegetables and effective in preventing many chronic diseases (Miller et al. 2000; Adom & Liu 2002; Ryan et al. 2011). Consumption of whole-grain products shown to several health benefits such as the reduced risk of cancer, chronic diseases, and cholesterol-lowering effect (Liyana-Pathirana & Shahidi 2005; Gasztonyi et al. 2011; Ma et al. 2014). Dietary fiber is associated with natural antioxidants and reported that it could be metabolized in the colon and utilized by the microbial microbiota for providing health benefits for humans (Xu et al. 2021). Genotype, environment or the interaction and individual environment parameters (temperature stress, precipitation, and drought level) can significantly affect antioxidant properties of wheat grain (Moore et al. 2006; Lv et al. 2013). As well as environmental and climate conditions grain structure and chemical properties of wheat may vary widely by agronomic treatments such as N fertilizer, organic/conventional farming system, pesticide application, and variety growth habit (long/short straw, winter/spring type). As the researchers mentioned that use of nitrogen fertilization, herbicides, and modern short straw varieties can all have a negative effect on the antioxidant content of wheat (Wang et al. 2020). Phenolic content has been found higher in organic growing condition than conventional farming system. Growing conditions and variety-specific properties have a certain effect on the biosynthesis and accumulation of phenolic acids. Total phenolic content of spring wheat varieties grown in organic conditions had higher than that of the wheat grown in conventional conditions (Vaher et al. 2010). Some studies have focused on the relationship between phenolic content or antioxidant activity with grain color and size. Ma et al. (2014a) reported that highly significant genotypic differences were observed in phenolic content and antioxidant activity among wheat varieties which are classified as black wheat with the highest antioxidant activity followed by red then white grain color wheat. However, antioxidant capacity and phenolic compounds in bran and flour highly depend on milling processes and products. Wheat grain parts such as the aleurone layer has the highest antioxidant activity wheat fraction, followed by the bran (Žilić 2016). Antioxidant activity of wheat flour extracts varies with cultivar and environmental conditions. Therefore, wheat variety might be the primary consideration for the enhanced antioxidant activity of wheat products. (Iqbal et al. 2007; Okarter et al. 2010; Tian & Li 2018). Different environmental conditions affect the phenol and antioxidant activity of wheat, and each antioxidant may respond to the environmental changes differently (Zhou & Yu 2004).

Besides contribution to health, amino acids of wheat, as a part of daily intake of amino acids constitute the importance of daily nutrition. Both in humans and animals nutrient quality is determined by protein content and the balance of amino acids in wheat grain. However, essential amino acids can not be synthesized by animals and in our bodies and hence must be provided in the diet (Shewry 2009; Zhang et al. 2017). The amino acid distribution of wheat is quite unbalanced and protein quality is associated with lacking essential amino acids like lysine, threonine, and methionine (Siddiqi et al. 2020). Lysine is considered the most important and limiting essential amino acid in cereal grains (Ufaz & Galili 2008; Konvalina et al. 2011). The nutritional quality can be improved by increasing protein content and limiting amino acids especially lysine. Due to the inverse relationship between lysine and protein content, a big challenge occurs so lysine can be used as a measure of protein quality (Anjum et al. 2005). Among the few studies that have been done about antioxidant and amino acid content of wheat grain, it is the first result of varieties grown in Turkey. In this study, we investigated the nutrient quality (protein and essential amino acid) and antioxidant properties of wheat varieties commonly grown in Turkey to facilitate the selection of varieties to produce high-quality wheat in breeding and agronomic studies and to give new impulses to future wheat quality efforts.

2. Material and Methods

2.1. Wheat samples

Bread wheat varieties widely grown all-around of Turkey were obtained from Trakya Agricultural Research Institute (Thrace region), Bahri Dağdaş International Agricultural Research Institute (Central Anatolia Region), Field Crops Central Research Institute (Central Anatolia Region), Transitional Zone Agricultural Research Institute (Central Anatolia Region), Maize Research Institute (Marmara Region) and Eastern Mediterranean Agricultural Research Institute (Southeastern Anatolia Region). Forty-five bread wheat varieties are collected after harvesting and stored +4 °C for chemical analysis (Table 1). Wheat varieties were classified due to grain color, spike color and type, growth habit, kernel weight, and adapted climate classification are given in Table 2. Climate zones and growth habits of the varieties were determined based on Köppen-Geiger classification (Öztürk et al. 2017; Yılmaz & Çiçek 2018). According to the adapted growth climate conditions of varieties seven subclimate types (Bsk: arid, steppe and cold summer, Csa: temperate, dry and hot summer, Cfa: temperate, without dry season but hot summer, Dsa: cold, dry and hot summer, Dsb: cold, dry and warm summer, Dfa: cold, without dry season and hot summer, Dfb: cold, without dry season and warm summer) were detected for different regions of Turkey. The Central Anatolia region is described as; the largest subclimate type of B zone (cold, semi-arid climate with cold winters and dry summer). Type C climate is dominant in coastal regions (Thrace, Marmara, Aegean, and Southeastern Anatolia) described as; dry and hot summer without dry season but hot summer. The four subclimate types (Dsb, Dsa, Dfa, Dfb) belong to type D is identified as cold winter, dry and warm summer season observe in Central Anatolia, Black Sea and Eastern Anatolia.

Table 1- Origin and description of the wheat varieties

Variety	*Label*	*Breeder Institute/Company*	*Variety*	*Label*	*Breeder Institute/Company*
Adana 99	adn	Eastern Mediterranean ARI	Karatoprak	krtprk	Bahri Dağdaş International ARI
Ahmetağa	ahmt	Bahri Dağdaş International ARI	Kate A 1	katea	Eastern Mediterranean ARI
Ak 702	ak702	Transitional Zone ARI	Kıraç 66	kirac	Transitional Zone ARI
Aldane	aldne	Trakya ARI	Konya 2002	konya	Bahri Dağdaş International ARI
Alpu 01	alpu	Transitional Zone ARI	Kutluk 94	kutluk	Transitional Zone ARI
Altay 2000	altay	Transitional Zone ARI	Lütfübey	ltfby	Field Crops Central ARI
Altınbaşak	abasak	Eastern Mediterranean ARI	Mesut	mesut	Transitional Zone ARI
Bayraktar 2000	byrktr	Field Crops Central ARI	Momtchill	mmtchl	Maize Research Institute
Beşköprü	bskpru	Maize Research Institute	Müfitbey	mftby	Transitional Zone ARI
Bezostaja-1	bztja	Maize Research Institute	Nacibey	ncby	Transitional Zone ARI
Ceyhan 99	cyhn	Eastern Mediterranean ARI	Osmaniyem	osmny	Eastern Mediterranean ARI
Çukurova-86	ckrova	Eastern Mediterranean ARI	Pamukova 97	pmkov	Maize Research Institute
Dağdaş 94	dagdas	Bahri Dağdaş International ARI	Pandas	pandas	Eastern Mediterranean ARI
Demir 2000	demir	Field Crops Central ARI	Sagittario	sgttario	Tasaco Tarım Company
Doğankent-1	dgnknt	Eastern Mediterranean ARI	Selimiye	slmy	Trakya ARI
Ekiz	ekiz	Bahri Dağdaş International ARI	Seri 2013	seri13	Eastern Mediterranean ARI
Gerek 79	gerek	Transitional Zone ARI	Seri 82	seri82	Eastern Mediterranean ARI
Gökkan	gokkan	Eastern Mediterranean ARI	Seyhan 96	syhn	Eastern Mediterranean ARI
Hanlı	hanlı	Maize Research Institute	Sönmez 01	snmz	Transitional Zone ARI
Harmankaya	hrmnkya	Transitional Zone ARI	Tahirova	throva	Maize Research Institute
İkizce	ikizce	Field Crops Central ARI	Tosunbey	tsnby	Field Crops Central ARI
İzgi 01	izgi	Transitional Zone ARI	Yüreğir 89	yrgir	Eastern Mediterranean ARI
Karahan 99	krhn	Bahri Dağdaş International ARI			

ARI: Agriculture Research Institute

2.2. Extraction of wheat samples

Wheat samples were milled in UDY Corporation, USA (Cyclone sample mill) miller and whole wheat flour was used for the determination of total phenolic content, free radical scavenging activity, and fiber content. Methanol extracts were prepared adding 50 mL methanol to 5 g each sample and shaked for 30 min. After shaking the methanolic extract was centrifuged at 5000 rpm for 20 min. The supernatants were collected and stored +4 °C until analysis (Ragaee et al. 2006).

2.3. Total phenolic contents

The total phenolic content of each whole wheat sample was determined by using the colorimetric method used by Kaluza et al. (1980) and Ragaee et al. (2006) and modified for the study. Briefly, extracts were reacted with Folin-Ciocalteu reagent and then neutralized with sodium carbonate. After 30 min. the phenolic mixture was centrifuged at 2000 rpm for 10 min. and was measured at 725 nm at the spectrophotometry. Gallic acid was used as the standard and phenolic content was expressed as µg gallic acid equivalents/g sample.

2.4. Total antioxidant activity

The total antioxidant activity was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method described by Brand-Williams et al. (1995). 3900 µl DPPH solution was added to 100 µl of methanolic wheat extract and the samples were put in 36 °C in water bath for 30 min. The absorbance of the mixture at 517 nm was measured and methanol was used as a blank, antioxidant activity was calculated as percent discoloration described below:

$$\text{AAC (Inhibition \%)} = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) \times 100/\text{Absorbance}_{\text{control}}$$

2.5. Crude fiber ratio

Whole milled wheat samples were scanned for crude fiber ratio (%) by using Near Infrared Reflected Spectroscopy (NIRS) method with Bruker MPA device (Oliveira & Franca 2011).

2.6. Crude protein content

Total protein content of flour samples was measured by CN elemental analyzer (Elementar-group, Hanau, Germany) using the high-temperature combustion method.

2.7. Essential amino acid analysis

Seven essential amino acids were identified by an amino acid analyzer (Biochrom 20 Plus, Biochrom Ltd. Cambridge, UK) in Berlin Freie Universität, Institute of Animal Nutrition and procedure chosen depends on the oxidation (for methionine and cysteine) and hydrolysis of samples (EU Commission Regulation 2009). In both oxidation and hydrolysis procedures, grain samples were ground to pass through a 0.8 mm (Perten Lab. Mill) sieve and flour sample (0.5 g) was added to a 100 mL bottle fitted with a screw cap. The samples placed in an ice-water bath and added 5 mL of oxidation mixture the left for 16 hours in refrigerator at 0 °C. After 16 hours samples removed from the refrigerator and decomposed the excess oxidation reagent by the addition of 0.84 g sodium disulfide. 25 mL of hydrolysis mixture (6 mol HCl/l containing 1 g phenol/l) was added to the oxidized samples and placed the bottle containing the mixture in an oven at 110 °C for 24 hours. On completion of hydrolysis, the mixture samples were removed and placed in an ice-water bath. After oxidation and hydrolysis procedure sample pH level adjusted to 2.20 at room temperature using sodium hydroxide (7.5 mol/l) solution and pH adjusted hydrolysate with sodium buffer to a 100 mL flask. Each sample was filtered through a 0.45 µm cellulose acetate membrane filter (Millipore) and injection volume was 200 µl.

Table 2- General characteristics and climate classification of the varieties

Variety	*Grain color*	*Spike type*	*Growth habit*	*Spike color*	*TKW (g)*	*Climate classification* (Köppen Geiger)*
Adana 99	White	Awned	Spring	White	28-39	Csa
Ahmetağa	Red	Awned	Winter	White	33.4	Bsk, Dsb, Dsa, Dfa
Ak 702	-	-	-	-	-	-
Aldane	Red	Awnedness	Spring	White	42.5	Cfa, Csa, Dsb
Alpu 01	White	Awned	Winter	White	40-44	Bsk, Dsb, Dsa, Dfa
Altay 2000	White	Awned	Winter	Brown	36-40	Bsk, Dsb, Dsa, Dfa
Altınbaşak	White	Awned	Spring	White	29.7-37.8	Csa
Bayraktar 2000	White	Awned	Winter	White	32-34	Bsk, Dsb, Dsa, Dfa
Beşköprü	Red	Awnedness	Winter	Red	37.2	Cfa, Dsb
Bezostaja-1	Red	Awnedness	Winter	White	38-44	Cfa, Bsk, Dsb, Dsa, Dfb
Ceyhan 99	Red	Awned	Spring	White	28-38	Csa, Cfa
Çukurova-86	-	-	-	-	-	-
Dağdaş 94	White	Awned	Winter	White	36-42	Bsk, Dsb, Dsa, Dfa
Demir 2000	Red	Awned	Spring	White	35.5	Bsk, Dsb, Dsa, Dfa
Doğankent-1	-	-	-	-	-	-
Ekiz	Red	Awned	Spring	White	36.4	Bsk, Csa, Cfa, Dsb, Dsa, Dfa,
Gerek 79	White	Awned	Winter	Brown	32-38	Bsk, Csa, Cfa, Dsa, Dsb, Dfa
Gökkan	White	Awned	Spring	White	35.7-40	Csa
Hanlı	Red	Awned	Spring	White	30-40	Csa
Harmankaya	Red	Awned	Winter	White	38-44	Bsk, Dsd, Dsa, Dfa
İkizce	Red	Awned	Winter	White	28-32	Bsk, Dsd, Dsa, Dfa
İzgi 01	White	Awned	Winter	White	36-42	Bsk, Dsd, Dsa, Dfa
Karahan 99	White	Awned	Winter	White	32-38	Bsk, Dsd, Dsa, Dfa
Karatoprak	White	Awned	Spring	White	32-40	Csa
Kate A 1	Red	Awnedness	Spring	White	35.9	Csa, Cfa
Kıraç 66	White	Awned	Winter	White	40.3	Bsk, Dsd, Dsa, Dfa
Konya 2002	Red	Awned	Winter	White	40-49	Bsk, Dsd, Dsa, Dfa
Kutluk 94	White	Awned	Winter	White	34-36	Bsk, Dsd, Dsa, Dfa
Lütfübey	Red	Awned	Winter	Brown	38	Bsk, Dsd, Dsa, Dfa
Mesut	Red	Awnedness	Winter	White	37-41	Bsk, Dsd, Dsa, Dfa
Momtchill	Red	Awnedness	Winter	White	42-45	Csa, Cfa
Müfitbey	White	Awned	Winter	White	38-42	Bsk, Dsd, Dsa, Dfa
Nacibey	Red	Awned	Winter	White	36-38	Bsk, Dsd, Dsa, Dfa
Osmaniyem	Red	Awned	Spring	White	35-42	Csa
Pamukova 97	Red	Awned	Spring	White	30-40	Csa
Pandas	Red	Awned	Spring	White	25.2-38.7	Csa
Sagittario	Red	Awned	Spring	White	40-45	Csa
Selimiye	Red	Awnedness	Winter	Red	38.5	Csa, Cfa
Seri 2013	White	Awned	Spring	White	36.2-40.9	Csa
Seri 82	-	-	-	-	-	-
Seyhan 96	White	Awned	Spring	White	40-42	Csa
Sönmez 01	White	Awnedness	Winter	White	38-44	Bsk, Dsd, Dsa, Dfa
Tahirova	White	Awned	Spring	White	34-46	Csa, Cfa
Tosunbey	White	Awned	Spring	White	30-35	Bsk, Dsb, Dsa, Dfa
Yüreğir 89	White	Awned	Spring	White	28.8-43.0	Csa

General characteristic information base on breeder institute/company. (-: lack of information)
Bsk: arid, steppe and cold summer, Csa: temperate, dry and hot summer, Cfa: temperate, without dry season but hot summer, Dsa: cold, dry and hot summer, Dsb: cold, dry and warm summer, Dfa: cold, without dry season and hot summer, Dfb: cold, without dry season and warm summer.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 19 statistical software (SPSS Inc. Chicago, USA). Data for each of the samples were evaluated by a one-way ANOVA procedure with 3 replications using the Duncan's multiple range test and linear correlation analysis were carried out to examine the relationships between the factors. Wheat varieties were differentiated using PC-ORD software through hierarchical clustering analysis (Mc Cune & Mefford 2006). Cluster analysis was performed using a tree diagram based on Euclidean distances was developed by Ward's method (Ward 1963; Arslan et al. 2019).

3. Results and Discussion

3.1. Total phenolic contents

Total phenolic content results indicate that statistically significant differences were obtained between varieties (P<0.01). Total phenolic content of the varieties ranged from 102.4 to 211.8 µg GAE/g (>2-fold) and the highest amount of phenolic content was measured in İzgi variety and the lowest in Sönmez variety. Values of varieties changed in a wide range and intensely between 140-200 µg GAE/g. In all evaluated varieties İzgi (211.8 µg GAE/g), Tahirova (209.0 µg GAE/g) and Demir 2000 (207.8 µg GAE/g) had the highest phenolic content and the lowest values obtained from Gökkan (135.0 µg GAE/g), Pamukova (130.4 µg GAE/g) and Sönmez (102.4 µg GAE/g), respectively (Table 3). A comparison of the total phenolic contents of varieties showed that İzgi, Demir 2000, Konya 2002, Osmaniyem, Ahmet ağa, Ceyhan 99, and Mesut varieties can be classified as highest total phenolic content whose grain color is red (Table 2). As mentioned by researchers grain color and bran fraction are related to phenolic content and antioxidant activity in wheat (Ma et al. 2014a; Žilić 2016). The results obtained by the previous studies are partially similar and have higher values compared to phenolic content results (Ragaee et al. 2006; Li et al. 2008; Revanappa & Salimath 2011) and also the widely ranging phenolic content may be due to different extracting methods (Engert & Honermeier 2011). It has been reported that phenolic content in wheat grain has positive contributions to human health and the antioxidant potential of wheat bran, a product in wheat milling industry emphasized by many studies. However, there are other categories of wheat products such as germ and shorts (Liyana-Pathirana & Shahidi 2006; Vaher et al. 2010).

3.2. Total antioxidant activity

Total antioxidant activity analysis of wheat grain based on DPPH radical scavenging activity and explained as % inhibition rate. As statistical results free radical scavenging activity of wheat grain changed by variety (P<0.01). The mean value of antioxidant activity was 17.19% and it varied from minimum 11.89% (Doğankent) to maximum 26.33% (Tosunbey) in all participated varieties. Besides Tosunbey variety, Momtchill (25.90%), Selimiye (23.78%), Ahmetağa (23.21%), Kutluk (23.13%), Nacibey (22.85%), Seri 82 (22.87%), Ceyhan 99 (21.99%) and Kıraç (21.60%) varieties have more than 20% inhibition value so these varieties more important in terms of antioxidant activity among other varieties (Table 3). The other 37 bread wheat varieties got 11-19% inhibition values. According to cluster analysis results (Figure 2) varieties with high antioxidant activity (ranged between 15.22% and 26.3%) are mainly located in Group C and has winter growth habit as described in Table 2. From these results, it can be concluded that growth habit of variety and growing conditions have a certain effect on the accumulation and content of the phenolic compounds (Vaher et al. 2010). Total antioxidant activity values are higher than results from several previous studies (Mpofu et al. 2006; Ryan et al. 2011). These findings could explain differences in grain phenol and antioxidant activity mainly change by genotype. Although environmental factors play an important role in antioxidant activity of cereals, genetic component is a key element with high heritability values to obtain higher antioxidant values (Lachman et al. 2012).

3.4. Crude fiber content

The primary components of dietary fiber of wheat are non-starch polysaccharides including insoluble dietary fiber and soluble dietary fiber. Health benefits attributed to the antioxidant capacity of phenolic and flavonoid compounds in cereals detected in crude fiber fractions (Sumczynski et al. 2015). Most bread products are made with refined white flours which are lack of important nutrients such as dietary fiber, vitamins, minerals and antioxidants due to removal of wheat bran and germ fractions (Xu et al. 2021). Wheat bran is a part of grain and milling product that has phenolic compounds mostly concentrated and may contribute to the total antioxidant activities of wheat. Most of the antioxidants in grains located in the bran and germ fractions (Miller et al. 2000). As obtained results, crude fiber mean value is 2.76% and highest fiber content obtained from Nacibey (3.30%) while minimum value noted in Mesut variety (2.25%). The fiber content of wheat grain remained limited (2-3% crude fiber content) and received not variable values but statistically differences observed between varieties (P<0.01). Apart from Nacibey in terms of crude fiber Gökkan (3.18%), Sönmez (3.25%), Karahan (3.03%), Ak 702 (3.18), Kıraç (3.17%) and Tosunbey (2.93%) varieties have come to the fore with high values (Table 3).

Table 3- Total phenolic, antioxidant activity, fiber, and protein content of varieties

Variety Name	*Phenolic content (µg GAE/g)*	*Antioxidant activity (DPPH %)*	*Fiber content (%)*	*Protein content (%)*
Adana 99	156.6^{ai}	$15.68^{d\text{-}j}$	$2.72^{e\text{-}j}$	13.00^{H*}
Ahmet Ağa	192.8^{af}	$23.21^{a\text{-}d}$	$2.44^{g\text{-}k}$	16.60^{j}
Ak 702	160.4^{ah}	$14.36^{g\text{-}j}$	3.18^{abc}	14.23^{z}
Aldane	164.8^{ah}	$15.30^{e\text{-}j}$	$2.66^{e\text{-}k}$	16.26^{o}
Alpu	173.9^{ah}	$18.82^{b\text{-}j}$	$2.45^{g\text{-}k}$	16.46^{l}
Altay	193.4^{af}	$15.91^{d\text{-}j}$	$2.64^{e\text{-}k}$	17.34^{h}
Altınbaşak	138.9^{ei}	$16.40^{c\text{-}j}$	$2.75^{d\text{-}j}$	13.24^{F}
Bayraktar	141.0^{di}	$14.38^{g\text{-}j}$	$2.86^{b\text{-}h}$	17.37^{g}
Beşköprü	176.9^{ah}	13.82^{hij}	$2.63^{e\text{-}k}$	12.99^{I}
Bezostaja 1	178.2^{ah}	$16.54^{c\text{-}j}$	$2.44^{h\text{-}k}$	17.34^{h}
Ceyhan 99	191.4^{ag}	$21.99^{a\text{-}g}$	$2.50^{f\text{-}k}$	13.43^{D}
Çukurova	153.7^{bi}	$19.87^{a\text{-}i}$	2.35^{jk}	12.83^{K}
Dağdaş 94	187.3^{ah}	$16.77^{c\text{-}j}$	$2.86^{b\text{-}i}$	15.10^{v}
Demir2000	207.8^{abc}	$16.48^{c\text{-}j}$	$3.03^{a\text{-}e}$	18.23^{d}
Doğankent	160.8^{ah}	11.89^{j}	$2.69^{e\text{-}j}$	12.76^{M}
Ekiz	153.6^{bi}	$16.06^{c\text{-}j}$	$2.51^{f\text{-}k}$	16.17^{q}
Gerek 79	187.5^{ah}	$15.07^{f\text{-}j}$	$2.82^{c\text{-}i}$	16.29^{n}
Gökkan	135.0^{ghi}	13.22^{ij}	3.18^{abc}	13.75^{B}
Hanlı	150.3^{ci}	$14.97^{f\text{-}j}$	$2.80^{c\text{-}i}$	13.01^{G}
Harmankaya	143.2^{di}	$15.64^{d\text{-}j}$	$2.71^{e\text{-}j}$	15.67^{r}
İkizce	166.4^{ah}	13.54^{ij}	$2.57^{f\text{-}k}$	14.50^{y}
İzgi	211.8^{a}	$15.22^{f\text{-}j}$	$2.72^{e\text{-}j}$	18.52^{c}
Karahan	137.9^{fi}	$16.08^{c\text{-}j}$	$3.03^{a\text{-}e}$	18.08^{e}
Karatoprak	183.2^{ah}	$14.75^{g\text{-}j}$	$2.54^{f\text{-}k}$	14.01^{A}
Kate A1	173.8^{ah}	$16.24^{c\text{-}j}$	$2.90^{a\text{-}f}$	16.43^{m}
Kıraç	178.3^{ah}	$21.60^{a\text{-}h}$	$3.17^{a\text{-}d}$	18.71^{b}
Konya	197.1^{ad}	$18.02^{c\text{-}j}$	$2.84^{b\text{-}i}$	16.66^{i}
Kutluk	195.9^{ae}	$23.13^{a\text{-}e}$	$2.75^{d\text{-}j}$	19.78^{a}
Lütfübey	189.0^{ag}	$19.29^{a\text{-}j}$	$2.82^{c\text{-}i}$	12.79^{L}
Mesut	194.4^{af}	$17.96^{c\text{-}j}$	2.25^{k}	16.51^{k}
Momtchill	189.1^{ag}	25.90^{ab}	$2.75^{d\text{-}j}$	11.99^{Q}
Müfitbey	155.5^{ai}	$16.02^{c\text{-}j}$	2.43^{ijk}	17.41^{f}
Nacibey	150.7^{ci}	$22.85^{a\text{-}f}$	3.30^{a}	16.25^{p}
Osmaniyem	195.0^{af}	$15.42^{d\text{-}j}$	$2.92^{a\text{-}f}$	14.96^{w}
Pamukova	130.4^{hi}	13.93^{hij}	$2.87^{b\text{-}g}$	12.35^{O}
Panda's	162.8^{ah}	13.35^{ij}	$2.90^{a\text{-}f}$	14.80^{x}
Sagittario	144.3^{di}	$17.07^{c\text{-}j}$	$2.74^{d\text{-}j}$	13.55^{C}
Selimiye	186.2^{ah}	23.78^{abc}	$2.56^{f\text{-}k}$	15.61^{s}
Seri 2013	175.5^{ah}	$14.75^{g\text{-}j}$	$2.87^{b\text{-}h}$	15.15^{u}
Seri 82	182.2^{ah}	$22.78^{a\text{-}f}$	$2.63^{e\text{-}k}$	12.39^{N}
Seyhan 95	190.3^{ag}	$15.48^{d\text{-}j}$	$2.65^{e\text{-}k}$	12.14^{P}
Sönmez	102.4^{i}	$16.80^{c\text{-}j}$	3.25^{ab}	15.34^{t}
Tahirova	209.0^{ab}	12.79^{ij}	$2.89^{b\text{-}f}$	12.39^{J}
Tosunbey	187.9^{ag}	26.33^{a}	$2.93^{a\text{-}f}$	16.60^{j}
Yüreğir	159.6^{ah}	13.93^{hij}	$2.76^{c\text{-}j}$	13.25^{E}
ANOVA	**	**	**	**
Std. Deviation	30.88	4.56	0.34	13.03
Maximum	211.8	26.33	3.30	19.78
Minimum	102.4	11.89	2.25	11.99
Average	171.4	17.19	2.76	15.16

*: the letters shown with the big font are the continuation of the alphabet, **: significance at $P<0.01$

3.5. Crude protein content

Most studies showed approximately 3-fold variation in protein content (from 7-22%) with about one-third of this being under genetic control (Shewry 2009). Grain quality is a complex of physical and chemical characteristics and affected by mainly genotype, environmental conditions and their interactions (Erekul et al. 2012). Crude protein values ranged between 11.99-19.78% that can be expressed as high protein content. The highest protein value was obtained from Kutluk variety which belongs to Central Anatolian Region and the lowest protein ratio was obtained from Momtchill variety. Karahan (18.08%), İzgi (18.52%) and Kıraç (18.71%) varieties also come to the forefront with high protein level (18%) between all evaluated varieties (Table 3). In breeding studies, there is a big challenge for breeders that there is found to be dilution effect between protein content and grain yield (Erekul & Köhn 2006). This inversely effect may cause to result high protein ratio that obtained from low yield potential varieties. Wheat grain yield value is low (approx. 2744 kg/ha) in Turkey compared to the European countries due to the variability in climate conditions of regions (Faostat 2018). Heat stress during grain filling period can cause a decrease of thousand-grain weight which means lower yield values that could be an explanation of high protein content. Therefore, regional weather conditions have a great impact on protein content due to the wheat yield. Turkish Grain Board (TMO) determines quality standards and purchases prices of farmers so 14% standard set in protein content for bread wheat grain. In our study, 28 varieties were found above this standard value and described as high protein content grains and differentiated as Group C in cluster dendrogram (Figure 2.) which are mostly adapted to winter growth habit in Central Anatolia Region (Bsk, Dsd, Dsa, Dfa sub climate type) described as arid and cold winter with dry and hot summer climate in Table 2.

3.6. Essential amino acid content

Wheat varieties showed a uniform essential amino acid profile that was characterized by lower concentrations of threonine, lysine, and methionine, and leucine obtained the highest average value between essential amino acids. While protein is major quality emphasis in wheat, it also enhances nutritional value of grain. Kutluk variety has higher threonine (0.491 g/100 g), valine (0.761 g/100 g), isoleucine (0.633 g/100 g), leucine (1.167 g/100 g), phenylalanine (0.896 g/100 g), lysine (0.464 g/100 g) and methionine (0.344 g/100 g) values than other varieties. Selimiye had remarkably high values from threonine (0.546 g/100 g), valine (0.802 g/100 g), isoleucine (0.876 g/100 g), phenylalanine (1.021 g/100 g), and lysine (1.475 g/100 g) amino acids. Statistically significant differences were observed ($P<0.01$) according to varieties and obtained essential amino acid values widely changed by genotypic variation (Table 4). Especially in terms of Selimiye lysine content, maximum value is (approx.) 5 times higher than minimum value but this situation was not seen for threonine and methionine amino acids. Higher leucine content made difference with highest average value compared to other essential amino acids. Therefore, varieties with high leucine content (İzgi, Karahan, Kıraç, Kutluk and Müfitbey) come to the forefront with high total essential amino acid content (Figure 1). These results extend our knowledge of high protein content caused an increase in the amount of amino acids. Concentrations of protein and essential amino acids were found significantly higher in winter growth habit compared to spring growth habit varieties and tree diagram of cluster analysis indicate that varieties in Group C have more nutritional value than others (Figure 2). Therefore, there is evidence that amino acid content of wheat grain basically depending on the protein content of variety also affected by growing conditions, climate and environment (Anjum et al. 2005; Konvalina et al. 2011).

Table 4- Essential amino acid content of varieties (g/100 g flour)

Variety name	*Threonine*	*Valine*	*Isoleucine*	*Leucine*	*Phenylalanine*	*Lysine*	*Methionine*
Adana 99	0.353^{t}	0.432^{J}	0.295^{F}	0.769^{C}	0.536^{B}	0.345^{xy}	0.228^{D}
Ahmet Ağa	0.389^{n}	0.496^{A}	0.366^{w}	0.893^{r}	0.660^{p}	0.370^{p}	0.281^{n}
Ak 702	0.382^{o}	0.508^{vw}	0.384^{s}	0.847^{v}	0.605^{x}	0.362st	0.260^{uv}
Aldane	0.443^{g}	0.523^{p}	0.380^{t}	0.983^{g}	0.733^{g}	0.399^{k}	0.235^{C}
Alpu	0.402^{l}	0.499^{z}	0.390^{q}	0.888^{t}	0.620^{v}	0.372^{o}	0.261^{tu}
Altay	0.405^{l}	0.504^{x}	0.365^{wx}	0.952^{k}	0.689^{m}	0.370^{p}	0.290^{l}
Altınbaşak	0.332^{y}	0.429^{K}	0.308^{C}	0.728^{IJ}	0.508^{I}	0.316^{E}	0.240^{B}
Bayraktar	0.413^{k}	0.553^{n}	0.420^{n}	0.951^{kl}	0.706^{k}	0.377^{n}	0.325^{e}
Beşköprü	0.346^{w}	0.413^{N}	0.275^{I}	0.729^{I}	0.513^{G}	0.344^{y}	0.264^{r}
Bezostaja 1	0.399^{m}	0.510^{tu}	0.404^{o}	0.928^{m}	0.664^{o}	0.370^{p}	0.293^{k}
Ceyhan 99	0.332^{y}	0.406^{O}	0.281^{G}	0.715^{L}	0.511^{H}	0.328^{B}	0.259^{v}
Çukurova	0.341^{x}	0.451^{G}	0.309^{C}	0.766^{D}	0.526^{D}	0.330^{A}	0.220^{F}
Dağdaş 94	0.348^{uv}	0.464^{E}	0.336^{A}	0.827^{y}	0.598^{y}	0.346^{xy}	0.264^{r}
Demir2000	0.434^{h}	0.571^{k}	0.448^{j}	1.003^{f}	0.710^{j}	0.401^{j}	0.313^{f}
Doğankent	0.350^{tu}	0.436^{I}	0.299^{E}	0.752^{E}	0.530^{C}	0.361^{t}	0.246^{A}
Ekiz	0.389^{n}	0.490^{B}	0.357^{z}	0.890^{s}	0.639^{t}	0.354^{w}	0.310^{gh}
Gerek 79	0.390^{n}	0.561^{m}	0.442^{k}	0.905^{n}	0.642^{s}	0.376^{n}	0.311^{g}
Gökkan	0.357^{s}	0.514^{rs}	0.384^{s}	0.802^{A}	0.553^{A}	0.361^{t}	0.267^{q}
Hanlı	0.331^{y}	0.456^{F}	0.337^{A}	0.748^{F}	0.485^{K}	0.337^{z}	0.247^{zA}
Harmankaya	0.366^{r}	0.507^{w}	0.387^{r}	0.837^{w}	0.585^{z}	0.365^{r}	0.309^{h}
İkizce	0.364^{r}	0.548^{o}	0.425^{m}	0.852^{u}	0.585^{z}	0.387^{l}	0.239^{B}
İzgi	0.464^{d}	0.692^{c}	0.553^{c}	1.085^{b}	0.808^{d}	0.464^{b}	0.374^{a}
Karahan	0.453^{f}	0.610^{f}	0.484^{h}	1.018^{e}	0.725^{h}	0.432^{d}	0.333^{c}
Karatoprak	0.367^{r}	0.488^{C}	0.377^{u}	0.812^{z}	0.553^{A}	0.326^{C}	0.283^{m}
Kate A1	0.443^{g}	0.549^{o}	0.405^{o}	0.981^{h}	0.739^{f}	0.407^{i}	0.327^{d}
Kıraç	0.459^{e}	0.645^{d}	0.518^{e}	1.020^{d}	0.757^{e}	0.441^{c}	0.301^{i}
Konya	0.424^{j}	0.567^{l}	0.438^{l}	0.967^{j}	0.698^{l}	0.373^{o}	0.296^{j}
Kutluk	0.491^{b}	0.761^{b}	0.633^{b}	1.167^{a}	0.896^{b}	0.464^{b}	0.344^{b}
Lütfübey	0.334^{y}	0.502^{y}	0.373^{v}	0.742^{G}	0.492^{J}	0.367^{q}	0.262st
Mesut	0.406^{l}	0.602^{h}	0.487^{g}	0.950^{l}	0.651^{q}	0.410^{h}	0.278^{o}
Momtchill	0.346^{w}	0.512^{s}	0.385^{s}	0.772^{B}	0.529^{C}	0.385^{m}	0.252^{x}
Müfitbey	0.473^{c}	0.598^{i}	0.449^{ij}	1.068^{c}	0.816^{c}	0.441^{c}	0.311^{g}
Nacibey	0.446^{g}	0.644^{d}	0.522^{d}	0.978^{i}	0.709^{j}	0.417^{f}	0.293^{k}
Osmaniyem	0.429^{i}	0.606^{g}	0.490^{f}	0.903^{o}	0.644^{r}	0.413^{g}	0.277^{o}
Pamukova	0.350^{tu}	0.509^{tu}	0.389^{q}	0.767^{D}	0.520^{E}	0.363^{s}	0.254^{w}
Panda's	0.391^{n}	0.582^{j}	0.450^{i}	0.888^{t}	0.626^{u}	0.433^{d}	0.267^{q}
Sagittario	0.331^{y}	0.445^{H}	0.336^{A}	0.740^{H}	0.515^{F}	0.300^{G}	0.253^{wx}
Selimiye	0.546^{a}	0.802^{a}	0.876^{a}	0.899^{p}	1.021^{a}	1.475^{a}	0.263^{rs}
Seri 2013	0.397^{m}	0.522^{p}	0.364^{x}	0.895^{q}	0.673^{n}	0.408^{i}	0.271^{p}
Seri 82	0.317^{z}	0.417^{M}	0.306^{D}	0.663^{N}	0.467^{L}	0.307^{F}	0.217^{G}
Seyhan 95	0.308^{A*}	0.424^{L}	0.313^{B}	0.669^{M}	0.456^{M}	0.338^{z}	0.225^{E}
Sönmez	0.373^{p}	0.513^{rs}	0.396^{p}	0.835^{x}	0.616^{w}	0.357^{v}	0.268^{q}
Tahirova	0.342^{x}	0.475^{D}	0.361^{y}	0.727^{K}	0.509^{I}	0.359^{u}	0.249^{y}
Tosunbey	0.443^{g}	0.621^{e}	0.490^{f}	0.978^{i}	0.720^{i}	0.420^{e}	0.248^{yz}
Yüreğir	0.349^{uv}	0.621^{e}	0.257^{H}	0.716^{L}	0.515^{F}	0.321^{D}	0.220^{F}
ANOVA	**	**	**	**	**	**	**
Std. Deviation	0.052	0.087	0.105	0.118	0.118	0.167	0.035
Maximum	0.546	0.802	0.876	1.167	1.021	1.475	0.374
Minimum	0.306	0.406	0.275	0.663	0.456	0.390	0.217
Average	0.390	0.532	0.405	0.866	0.627	0.399	0.273

*: the letters shown with the big font are the continuation of the alphabet, **: significance at P<0.01

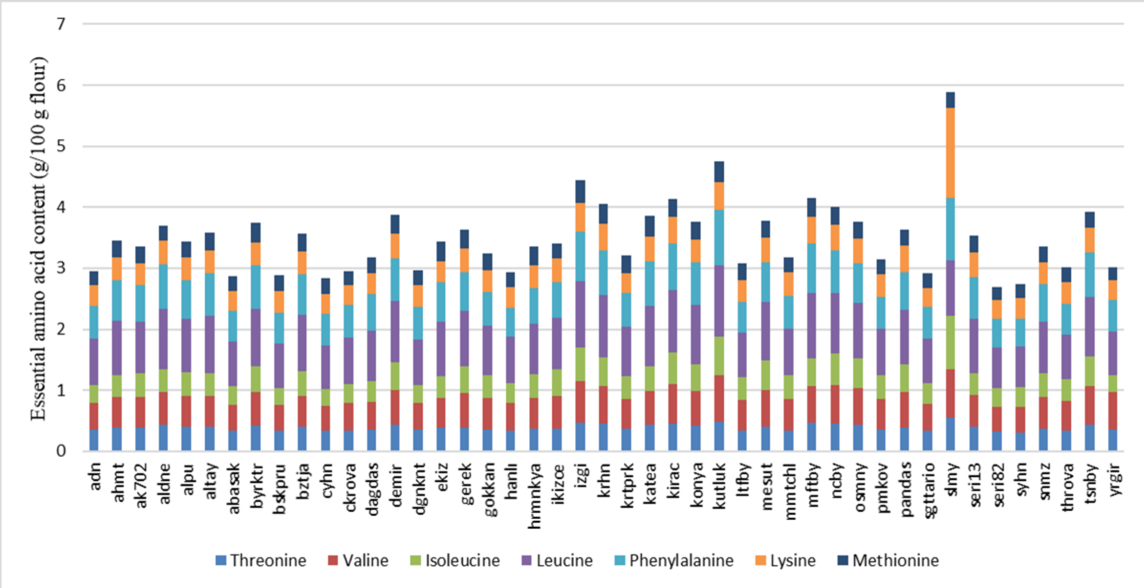

Figure 1- Essential amino acid distribution of varieties (g/100 g flour)

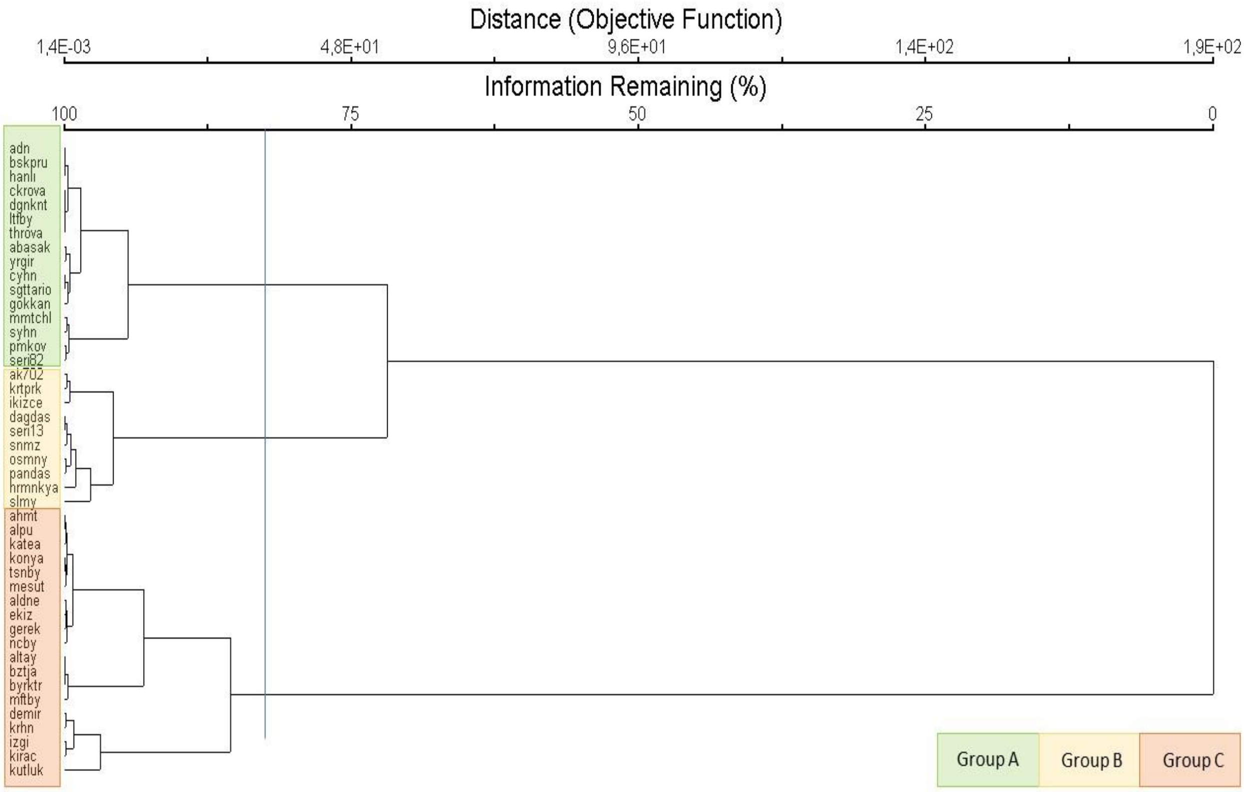

Figure 2- Dendrogram of cluster analysis for 45 varieties based on antioxidant and essential amino acid content

3.7. Correlation and cluster analysis of parameters

Phenolics are known as the most significant bioactive compounds with high antioxidant activity value in wheat grain (Žilić 2016). In this respect, it was found that total antioxidant activity was influenced by the phenolic content of grain in this study. The analysis showed a positive significant correlation (r= 0.196**) between total antioxidant activity and total phenol content of wheat grain. On the other side crude fiber content also had a significant correlation (r= 0.171**) with total phenolic content of grain. This result is also supported by few studies that bran fraction has many health-beneficial compounds such as vitamins, fiber, and whole-grain cereals. Bioactive compounds are mainly concentrated outer layer of grain and potentially health-beneficial compounds such as phenolics are mostly found in the aleurone layer (Mazzoncini et al. 2015; Serea & Barna 2011; Žilić et al. 2012). However, amino acid content of wheat grain is positively related to protein content that showed a strong correlation between crude protein and essential amino acids (Table 5). Grain protein concentration depends on the combination of many factors as well as amino acid concentration affected by protein exchange. In our study, we also found a positive correlation between total antioxidant activity and essential amino acids (except leucine and methionine) this could be important from a nutritional physiology perspective. Dendrogram (Figure 2) showed that the 45 wheat varieties were gathered into three main clusters (Group A, Group B and Group C) taking into existing similarities for antioxidant and amino acid properties. The first cluster (Group A) comprised mainly spring wheat varieties (Adana 99, Hanlı, Tahirova, Altınbaşak, Yüreğir, Ceyhan 99, Sagittario, Gökkan, Seyhan 96, Pamukova) and three winter wheat varieties (Beşköprü, Lütfübey and Momtchill). The second cluster (Group B) comprised both spring wheat varieties (Karatoprak, Seri 2013, Osmaniyem, Pandas) and winter wheat varieties (İkizce, Dağdaş 94, Sönmez 01, Harmankaya, Selimiye). The third cluster (Group C) comprised mainly 14 winter wheat varieties (Ahmet ağa, Alpu 01, Konya 2002, Mesut, Gerek 79, Nacibey, Altay 2000, Bezostaja-1, Bayraktar 2000, Müfitbey, Karahan 99, İzgi 01, Kıraç 66, Kutluk) and 5 spring wheat varieties (Kate A1, Tosunbey, Aldane, Ekiz, Demir 2000). Group A is differentiated by spring wheat varieties mainly adapted to dominant subtropical semihumid and humid coastal climate type of Turkey recognized as tempered, dry and hot summer (Csa climate classification in Table 2) while third cluster group comprised mainly winter wheat varieties mainly adapted to arid and cold winter with dry and hot summer climate type seen in Central Anatolia Black Sea and Eastern Anatolia regions (Table 2). According to these hierarchical associations, it could be confirmed that antioxidant and amino acid properties are related to mainly growth habit (spring vs winter) and genetic variability.

Table 5- Linear correlation (r) of the parameters studied

	Aac	*Fiber*	*Pro*	*Thr*	*Val*	*IsoLeu*	*Leu*	*PheAl*	*Lys*	*Meth*
Phe	0.196**	0.171*	0.150*	0.151*	0.152*	0.164*	0.148*	0.154*	0.111	0.118
Aac		0.060	0.136	0.215**	0.226**	0.286**	0.137	0.227**	0.233**	-0.008
Fiber			0.010	-0.025	-0.014	-0.014	0.002	-0.013	-0.023	0.003
Pro				0.817**	0.662**	0.584**	0.943**	0.824**	0.206**	0.805**
Thr					0.868**	0.856**	0.887**	0.977**	0.625**	0.633**
Val						0.905**	0.745**	0.848**	0.629**	0.534**
IsoLeu							0.657**	0.857**	0.810**	0.483**
Leu								0.873**	0.247**	0.783**
PheAl									0.663**	0.642**
Lys										0.111

Phe: Total phenolic content; Aac: Total antioxidant activity; Pro: Protein; Thr: Threonine; Val: Valine; IsoLeu: Isoleucine; Leu: Leucine; PheAl: Phenylalanine; Lys: Lysine; Meth: Methionine. Significance at $P<0.05$ (*) and $P<0.01$ (**)

4. Conclusions

To our knowledge, there are only a few studies published on the phenol, antioxidant, and amino acid potential of wheat. Thus, the first data of wheat grain about health and nourishment value obtained and aimed to be used in terms of further food and agriculture studies by scanning widely grown Turkish varieties. The results of the present study indicate that antioxidant activity and amino acid content of wheat grain mainly change by genotype. In evaluated varieties Ceyhan 99, Ahmetağa, Kutluk, Momtchill, Selimiye and Seri 82 had higher values in terms of phenolic content and antioxidant activity. Kutluk and Selimiye varieties exhibited high essential amino acid profile and in terms of protein quality to any other varieties. Central Anatolia stands out in terms of varieties with high protein and essential amino acid content across all regions. Clustering analysis also underlined varieties with winter growth habit as higher nutritional value compared to spring growth habit varieties. In correlation results the most attractive result is increasing fiber content promotes higher phenolic content and almost all essential amino acids increased as the protein of the wheat grain increased. Genotypical variation was found to be an important factor to achieve healthy and nutritionally superior wheat and its products can be evaluated in breeding studies.

By the research, it is tried to be revealed a new approach with interdisciplinary study about essential amino acid, phenol and antioxidant properties of bread wheat and thus obtained new results revealed the strength of relationships between parameters.

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