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Determination of protein, vitamins, amino acids and mineral element content of Yenice and Pınarlı bean (*Phaseolus vulgaris* L.) genotypes

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ABSTRACT

In this study, yield components (ash, carbohydrates, crude fibre, energy, protein and sucrose), vitamins (retinol, thiamine, riboflavin, niacin, pyridoxine, folate and Ldehydroascorbic acid), macro-micro nutrients (phosphorus, potassium, magnesium, calcium, sodium, zinc and iron) and amino acids (alanine, proline, arginine, aspartic acid, methionine, glutamic acid and phenylalanine) of two bean genotypes (*Phaseolus vulgaris* L. cvs. Yenice and Pinarli) were investigated. Yenice and Pinarli bean genotypes (*Phaseolus vulgaris* L.) commonly grown in Çamoluk region in Central Black Sea Region of Turkey were used as the plant material of this study. Significant differences were observed in ash, crude fibre, lipids, moisture, protein, salt and sucrose content of bean genotypes. While sucrose (4.56 g 100 g^{-1}), salt (0.12 g 100 g^{-1}) carhydrates (38.41 g 100 g^{-1}) and crude fiber (25.57 g 100 g^{-1}) values were higher in Pinarli , lipids (1.69 g 100 g^{-1}) and protein 21.72 g 100 g^{-1}) contens were higher in Yenice genotype. Similarly, vitamin levels were also significantly different. Thiamine (0.50 mg 100 g^{-1}), niacin (3.82 mg 100 g^{-1}), retinol (4.15 µg 100 g^{-1}), riboflavin (0.16 mg 100 g^{-1}), pyridoxine (0.33 µg 100 g^{-1}) and folate (234.50 µg 100 g^{-1}) contents of Pinarli genotype were significantly higher than Yenice. Potassium content (16145.0 mg kg^{-1}) of Yenice genotype and phosphorus content (8349.1 mg kg^{-1}) of Pinarli genotype were also found statistically significantly. Except for tryptophan and phenylalanine, significant differences were also observed in amino acids of bean genotypes. Amino acid content of Pinarli genotype was also higher than Yenice genotype.Both genotypes were especially rich in aspartic acid, lysine and glutamic acid.

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1. Introduction

Among the edible legumes, beans have the first place in production sites and amount of production worldwide 26.833.000 t (FAO, 2017). Although beans are mostly preferred as dry legume, they are also consumed as fresh, canned and pickled. Especially dry beans with high protein, vitamin, carbohydrate, amino acid, energy, fibre, mineral and other nutrient content are commonly consumed by humans to meet daily nutritional needs (Kutoš et al., 2003). Dry legumes including beans have various physiological impacts in treatment of coronary heart disease, diabetes, intestinal diseases, prostate, stomach diseases and some types of cancers (Hughes et al., 1997; Tharanathan and Mahadevamma, 2003; Preuss, 2009). Especially the slow digestible carbohydrate content plays important roles in reducing glycemic index of the diet and risk of myocardial infarct (Bazzano et al., 2001; Kabagambe et al., 2005; Helmstädter, 2010). American Diabetes Association (ADA) also recommended ample consumption of dry beans in daily diets for treatment of metabolic disorders, to prevent excessive weight gains and to maintain body form (Siddiq, 2010).

Nutrient accumulation, especially protein and carbohydrate accumulation in beans is occurred in seed tissues during pod development period (Duranti and Gius, 1997). Dry beans contain about 15-25% protein and 55-65% carbohydrate (Bravo et al., 1999; Bednar et al., 2001; de Almeida Costa et al., 2006; Mallillin et al., 2008). Besides supplying sufficient protein, legumes are also a well mineral and vitamin source (Amarowicz and Pegg, 2008). They contain high amounts of iron and zinc. Beans are also a rich folic acid source and meet about 90-95% of daily need. There is an inverse relationship between folic acid intake and colon cancer (Giovannucci et al., 1998). Dry beans also contain thiamine, riboflavin, niacin, vitamin B12 and folic acid, and cooked bean preserves about 70-75% of water-soluble vitamins (Augustin, 1981).

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Just because of high adaptation capabilities, legumes exhibit several genotypic and phenotypic diversities. Beside genetics, geographical position, growing seasons and environmental factors play important roles in nutrient content of dry beans (Sathe et al., 1984; Florez et al., 2009). Depending on those factors, physical characteristics and nutrient composition of seeds may greatly vary. Turkey is the gene source of several plant species (Güner, 2001). With different geographical formations and climates, Turkey has various natural reserves with genetic diversity and wealth. Rapidly increasing population, developing technology, changes in consumption habits of people and the desire to gain more with less investment increased the use of high-yield improved breeds in production and ultimately resulted in rapid loss of genetic diversity and natural wealth.

Çamoluk region of Turkey have been known with bean cultivation lands for many years. Yenice and Pinarli genotypes are commonly cultivated in the region. However, there isn't any information in literature about ash, carbohydrates, crude fibre, energy, protein, vitamin, nutrients and amino acid content of these genotypes. The present study was conducted to determine the nutritional properties and to widespread the production of these genotypes.

2. Materials and methods

2.1. Plant material

Yenice and Pinarli bean genotypes (*Phaseolus vulgaris* L.) commonly grown in Çamoluk region (40°.06'.40.88" N latitude, 38° 53' 29.08" E longitude and 1.200 m altitude) in Central Black Sea Region of Turkey were used as the plant material of this study. The average annual precipitation of the growing sites is 548 mm. Regular agronomic practices required for bean culture were implemented. Beans were sown in May 2014 and manually harvested in September 2014 in Çamoluk. Harvested beans were air dried and residual materials were manually cleaned. Sampling was made from 200 kg of each genotype. Genotypes were divided into 3 lots each of 10 kg. Beans were preserved in polyethylene bags at 4 °C and 55±5% relative humidity in a cold storage for a month until the analyses. Then the samples were transferred to Food Industry Laboratory in Marmara Research Centre of Turkish Scientific and Technological Research Institute (TUBİTAK MAM–Kocaeli, Turkey) in aluminum boxes for analyses to be performed.

2.2. Proximate chemical composition analysis of dry beans

The 2 kg samples of each genotype were washed twice with deionized water. The samples were dried in an oven at 55 °C for 24 hours. Then the samples were grinded in an analytical mill. Atwater extended factor system was used to determine energy and carbohydrate contents of the samples (Merrill and Watt, 1973). Moisture content, crude ash analysis, salt contents, crude protein contents, oil content, water-soluble and non-water-soluble fibre and sugar component analysis were performed in accordance with the method recommended by Association of Official Analytical Chemists (AOAC, 2002). For moisture content determination seed samples were dried in an oven at 105 °C until constant weight, cooled down, weighed in a digital balance (±0.1 mg) and 3 g samples were taken from homogeneous samples in porcelain crucibles. These samples were then dried in an oven at 105 °C for 5 hours until constant weight. Finally, the dried samples were cooled in a desiccator until room temperature and weighed in a digital balance again. For crude ash analysis the samples, dried for moisture content, were placed in an ash oven and ashed at 550 °C for 5 hours (until having light grey colour) then cooled in a desiccator until room temperature and weighed. Crude protein contents were determined according to Kjeldahl method in accordance with the AOAC. Homogenized samples were subjected to etching in kjeldahl tubes. Following distillation and titration processes, crude protein content rations (%) were calculated. Oil content was determined with Soxhlet extraction method of AOAC. Water-soluble and non-water-soluble fibre analyses were performed by using enzymatic gravimetric method. Samples were analysed by using "MES/TRIS" buffer solution. Sugar components were analysed with chromatographic method. Monosaccharide (fructose, glucose) and disaccharide (sucrose, maltose, lactose) contents were determined by measuring the area of peaks obtained from high performance liquid chromatography (HPLC) refractive index (RI) detector.

2.3. Macro and micro element analyses

Mass spectrometry method was used in mineral analyses. Minerals were made soluble through closed system microwave wet etching and concentrations were determined by using ICP-OES. Sodium, potassium, phosphorus, calcium, magnesium iron and zinc contents of the bean genotypes were determined in accordance with AOAC (2002).

2.4. Vitamin analyses

Water soluble vitamins of retinol, thiamine, riboflavin, niacin, pyridoxine, folate and L-dehydroascorbic acid contents were determined in this study. Vitamin C content was determined as L-dehydroascorbic acid. Sample vitamin C contents were determined by measuring the area of peak obtained from HPLC UV detector following the sample extraction with methaphosphoric acid (Bognar, 1998). B vitamins were determined in HPLC HL detector following an enzymatic incubation (Gauch et al., 1992). Vitamin A was determined by measuring the area of peak received from reverse-phase analytical HPLC UV detector following direct extraction and placing the extract into proper dissolvent (Genestar and Grases, 1995).

2.5. Amino acid analyses

For amino acid composition, proteins were hydrolyzed into amino acid components by deriving with phenyl isothiocyanate in ultra-fast liquid chromatography (UFLC) and then area of peaks obtained from UFLC UV detector was measured.

2.6. Statistical data analyses

The Bartlett's test was used to assess the homogeneity of variances in different groups. If parametric test assumptions

were available, two independent group means were compared by Student's t-test. The results were expressed as the mean. Data analyses were performed with SPSS software (version 23.0, SSPS Inc, Chicago IL, USA). A p-value of <0.05 was considered as significant.

3. Results and discussion

Except for carbohydrates, significant differences were observed in chemical components of bean genotypes (Table 1). Yenice genotype had higher ash, energy, lipids, moisture and protein contents and Pinarli genotype had higher crude fibre, salt and sucrose contents. Protein content of Yenice genotype was 5.43% higher than the protein content of Pinarli genotype whereas Pinarli genotype had 16.45% higher sucrose content than Yenice genotype.

The genotypes investigated in this study were found to be rich in some chemical components (Siddiq, 2010). However, some components were found to be lower than the previously reported values in literature (Bravo et al., 1999). The genotypes, ecological conditions, growing conditions and altitude might have resulted in such differences in chemical components. Thusly, dependence of protein, ash, energy, lipids, moisture and sucrose-like components primarily on genotype, growing site, growing season, light intensity, day length, temperature, plant nutrition and irrigation practices were reported by several previous researchers (Mack and Singh, 1969; Shellie and Hosfield, 1991; Gonzalez et al., 2006; Kumar et al., 2006; Kinaci et al., 2008; Pereira et al., 2011). Ceyhan et al. (2012) reported that hot and dry conditions after a precipitated period increased protein content.

Table 1. Chemical components of different bean genotypes

Components	Bean genotypes		*T value*	*P value*
	Yenice	Pinarli		
Ash (g 100 g^{-1})	4.10	3.90	-48.99	0.000 ***
Carbohydrates (g 100 g^{-1})	36.22	38.41	1.81	0.145 ns
Crude fiber (g 100 g^{-1})	23.45	25.57	26.14	0.000 ***
Energy (kcal 100 g^{-1})	301.5	296.0	-5.21	0.006 **
Lipids (g 100 g^{-1})	1.69	1.04	-17.27	0.000 ***
Moisture (g 100 g^{-1})	10.83	10.53	-12.08	0.000 ***
Protein (g 100 g^{-1})	21.72	20.54	-15.68	0.000 ***
Salt (g 100 g^{-1})	0.06	0.12	40.93	0.000 ***
Sucrose (g 100 g^{-1})	3.81	4.56	22.28	0.000 ***

Significance *: p<0.05; **: p<0.01; ***; p<0.001; ns: no significance

Although oil content primarily depends on plant genetics, it was reported that high temperatures during seed formation positively affected oil contents and higher oil contents were also observed in beans grown at high altitudes and cold climates (Kimber and McGregor, 1995). Current oil contents were generally higher than the values reported by Shimelis and Rakshit (2005) for beans grown in Ethiopia, but lower than the values reported by El-Adway et al. (2003) for beans grown in Egypt.

Significant differences were observed in vitamin contents of investigated bean genotypes (Table 2). Thiamine and niacin content (p<0.05), retinol, riboflavin and pyridoxine content (p<0.01) and folate content (p<0.001) of Pinarli genotype were significantly higher than the values of Yenice genotype. Especially retinol content of Pinarli genotype was 107.5%, folate content 46% and pyridoxine content 37% higher than the values of Yenice genotype.

Table 2. Vitamin content of different bean genotypes

Bean genotypes		*T value*	*P value*	
Yenice	Pinarli			
Retinol (µg100 g^{-1})	2.00	4.15	20.48	0.000 **
Thiamine (mg100 g^{-1})	0.46	0.50	4.38	0.012 *
Ribofilavin (mg100 g^{-1})	0.15	0.16	5.99	0.004 **
Niasin (mg100 g^{-1})	3.52	3.82	4.48	0.011 *
Pyridoxine (mg100 g^{-1})	0.24	0.33	4.91	0.008 **
Folat (µg100 g^{-1})	160.00	234.50	28.38	0.000 ***
L-dehydroascorbic acid (mg100 g^{-1})	5.20	5.60	9.72	0.001 **

Significance *: p<0.05; **: p<0.01; ***; p<0.001; ns: no significance

Persistent vitamin deficiency may result in nervous system disorders, mental deficiency, Alzheimer disease, atherosclerotic cardiovascular diseases (Popescu and Golubev, 2012). Therefore, humans should have sufficient vitamins through their daily diets. Dry beans with high vitamin content are commonly preferred to meet such needs. Vitamin contents of Pinarli genotype were generally higher than both Yenice genotype of the present study and the values reported by Granito et al. (2002) and Zanovec et al. (2011). Folic acid plays an important role in cell division and cell genetic structure and it is an essential nutrient for nervous system development of a fetus throughout the early stages of pregnancy (Honein et al., 2001). A pregnant woman should consume average 400 µg folic acid daily throughout the pregnancy (Hewitt et al., 1992; Cornel and Erickson, 1997). Pinarli genotype with average 234 µg100 g-1 folic acid content may meets 60% of such a need by consuming daily 100 g beans. In this respect, Pinarli genotype was prominent as a rich folic acid source.

Significant differences were also observed in potassium and phosphorus contents of investigated genotypes ($p<0.0001$). With regard to micro elements, significant differences were observed only in Na contents of genotypes ($p>0.05$) (Table 3).

Table 3. Macro and micro mineral contents of different bean genotypes

Minerals (mg kg^{-1})	Bean genotypes		*T value*	*P value*
	Yenice	Pinarli		
Macro elements				
P	6917.3	8349.1	15.49	0.000 ***
K	16145.0	14730.0	-39.63	0.000 ***
Mg	1261.0	1275.5	0.49	0.649 ns
Ca	1468.0	1482.0	0.23	0.830 ns
Micro elements				
Na	54.46	68.56	4.37	0.012 *
Zn	29.56	30.55	0.75	0.493 ns
Fe	56.40	53.53	-2.15	0.098 ns

Significance *: $p<0.05$; **: $p<0.01$; ***; $p<0.001$; ns: no significance

In previous studies (Siddiq, 2010; Popescu and Golubev, 2012), K contents of dry beans were reported as between 390-700 mg 100 g^{-1}, P contents as between 310-510 mg 100 g^{-1}, mg contents as between 90-140 mg 100 g^{-1} and Na contents as between 1-15 mg 100 g^{-1}. Potassium content of both Pinarli and Yenice genotypes, phosphorus content of Pinarli genotype was found to be higher than the previously reported values (Devos, 1988; Beebe et al., 2000; Wang et al., 2010; Martinez Meyer et al., 2013). Ca, Na, Zn and Fe contents of investigated genotypes were complying with the previous literature values (Beebe et al., 2000). There is a significant relationship between genotypic difference and mineral matter content (Talukder et al., 2010). However, impacts of genotypic variances on mineral matters have not been physiologically elucidated, yet (Welch and Graham, 2002). The differences observed in the present study may resulted from different ecological conditions, soil textures, mineral matter levels of soil, plant root development and distribution of minerals in plant tissues.

Amino acid contents of investigated bean genotypes are provided in Table 4. While significant differences were not observed in tryptophan and phenylalanine contents of the genotypes the differences in alanine, threonine, proline, glutamic acid, tyrosine glycine, valine, leucine, isoleucine, serine, arginine, aspartic acid, methionine, lysine and histidine contents were found to be statistically significant. The present essential amino acid contents were higher than the values reported by Candella et al. (1997) for kidney beans and by Morales-de Leon et al. (2007) for black beans. Especially aspartic acid, valine and histidine values of the present genotypes were distinctively higher than the previously reported values. Growing conditions, nutritional status, genotypic differences, climate conditions and environmental conditions might be effective in having such different values. Oshodi et al. (1995) reported significant effects of growing conditions and genetic differences on amino acid composition of beans

4. Conclusions

Consumption of foods with rich nutritional values has various preventive impacts on diabetes, heart diseases and various cancer type. Protein contents of investigated genotypes varied between 21.72 - 20.54% and dietary fibre contents between 23.45 - 25.57%, in addition oil contents were low and cholesterol-free. The genotypes were highly rich in various vitamins (A, B, C), mineral matters (potassium, phosphorus and iron) and essential amino acids. Therefore, investigated genotypes may be recommended as a nutrient source in daily diets and potentially be used also in treatment of coronary heart disease, diabetes, intestinal diseases, prostate, stomach diseases and some cancer types.

Table 4. Amino acid contents of different bean genotypes

Amino acids	Bean genotypes		*T value*	*P value*
(mg100 g^{-1})	Yenice	Pinarli		Yenice
Alanine	839.0	872.0	3.57	0.023 *
Glycine	780.5	867.0	19.14	0.000 ***
Valine	1001.5	1054.5	10.78	0.000 ***
Leucine	1623.5	1697.5	21.22	0.000 ***
Isoleucine	977.5	1037.5	20.19	0.000 ***
Threonine	791.0	830.5	5.86	0.004 **
Serine	1159.0	1252.0	28.48	0.000 ***
Proline	816.5	869.5	5.28	0.006 **
Arginine	571.5	682.5	121.29	0.000 ***
Tryptophan	177.5	175.5	-0.39	0.716 ns
Aspartic acid	2591.0	2670.0	23.92	0.000 ***
Methionine	238.0	270.5	20.91	0.000 ***
Glutamic acid	3130.5	3280.0	5.96	0.004 **
Phenylalanine	1205.0	1210.5	1.25	0.280 ns
Lysine	2118.0	2567.0	43.47	0.000 ***
Histidine	577.0	590.5	11.34	0.000 ***
Tirozine	698.0	717.5	6.35	0.003 **

Significance *: p<0.05; **: p<0.01; ***; p<0.001; ns: no significance

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Disclosure statement

No potential conflict of interest was reported by the author

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