

Assessment of Peanut (*Arachis hypogaea* L.) Genotypes in Terms of Some Nutritional and Antioxidant Parameters

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Phenolics

Abstract: The objective of this study was to investigate some nutritional and antioxidant parameters of six different peanut genotypes grown in Turkey. Unshelled seed extracts were used for analyses. The skins of the seeds were not peeled. The crude protein and the crude oil amounts, fatty acid compositions, the total phenolic and flavonoid contents and the total antioxidant capacities were investigated. The crude oil contents of seeds ranged from 43.9 to 45.9%. On the other hand, the crude protein contents varied between 26.9 and 30.6%. The highest protein percentage was determined in NC-7 seeds. The crude oil contents of the genotypes were not different statistically. Oleic acid was the most plentiful fatty acid in all genotypes and followed by linoleic acid, palmitic, stearic and linolenic acids, respectively. The highest oleic acid/linoleic acid ratio was found in NC-7 and DA35/2011. Data obtained from the total phenolic and the total flavonoid analyses had similar manners for each genotype. NC-7 and DA35-2011 were the poorest genotypes in the total phenolic and flavonoid levels. With regards to the ABTS⁺ radical scavenging activity, the most powerful genotypes were Gazipaşa and Sultan. Our study showed that the more phenolic content gave rise to the more antioxidant capacity for each genotype.

Yerfıstığı (*Arachis hypogaea* L.) Genotiplerinin Bazı Besinsel ve Antioksidan Parametreler Bakımından Değerlendirilmesi

Anahtar Kelimeler

Antioksidan,
Arachis hypogaea,
Yağ asitleri,
Beslenme,
Yerfıstığı,
Fenolikler

Özet: Bu çalışmanın amacı Türkiye’de yetiştirilen altı farklı yerfıstığı genotipinin bazı besinsel ve antioksidan parametrelerini araştırmaktır. Analizler için kabuksuz tohum özütleri kullanılmıştır. Tohumların iç kabukları soyulmamıştır. Ham protein ve ham yağ miktarları, yağ asidi kompozisyonları, toplam fenolik ve flavonoid içerikleri ile toplam antioksidan kapasiteleri araştırılmıştır. Tohumların ham yağ içerikleri %43.9’dan %45.9’a kadar sıralanmıştır. Öte yandan, ham protein içerikleri %26.9 ile 30.6 arasında değişim göstermiştir. En yüksek protein yüzdesi NC-7 tohumlarında belirlenmiştir. Genotiplerin ham yağ içerikleri istatistiksel olarak farklı değildi. Oleik asit tüm genotiplerde en çok bulunan yağ asidi olmuş ve bunu sırasıyla linoleik asit, palmitik asit, stearik asit ve linolenik asit takip etmiştir. En yüksek oleik asit/linoleik asit oranı NC-7 ve DA35/2011’de gözlenmiştir. Toplam fenolik ve toplam flavonoid analizlerinden elde edilen veriler her bir genotip için benzerdir. NC-7 ve DA35-2011 toplam fenolik ve toplam flavonoid içerikleri açısından en fakir genotiplerdir. ABTS⁺ radikal süpürme aktivitesine göre, en güçlü genotipler Gazipaşa ve Sultan’dır. Çalışmamız, daha fazla fenolik içeriğin her bir genotip için daha fazla antioksidan kapasiteye yol açtığını göstermiştir.

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important legume predominantly grown in tropical, subtropical and

temperate climates [1, 2]. Global peanut production is nearly 43 million tonnes from the 25.4 million ha of agricultural lands. Turkey is one of the main peanut producers in the Mediterranean basin. Two-thirds of

peanut grown in the world is crushed for oil while the remaining is used for peanut butter, snack and confection production [3]. Dried peanut seeds contain 35-56% oil and 25-30% protein and 9.5-19.0% carbohydrate [4]. Peanut oil has pale yellow colour and the distinctive odour and aroma of peanut [5]. Oleic, linoleic, palmitic and stearic acids are main fatty acids in the oil content of peanut seeds. Oleic acid and linoleic acid constitute 75-80% of the total fatty acids in peanut [3]. Increasing of high oleic/linoleic acid ratio is a crucial indicator of shelf life and oil stability of peanut originated products [5]. It is widely recognized that nuts including peanut are rich in fat. However, fatty acid composition in these nuts has cardioprotective roles [6]. In addition to their valuable nutritional properties, peanut seeds have a plenty of phytochemical compounds which are essential for human health. Age- and diet-related human diseases are usually the results of cellular disorders arise from free radical formation in cells. Antioxidants as health-promoting natural bioactive compounds slow down or prevent the oxidation of cellular molecules [7]. Various methods have been used to evaluate antioxidant potentials of foods including ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) [8, 9]. The ABTS method relies on the decolourization of pre-generated blue ABTS radical cations (ABTS⁺) through the presence of antioxidants in samples.

Carcinogenicity and toxicity limits the preferability of synthetic antioxidants in food products [10]. Phenolic compounds possess strong antioxidant and radical scavenging capacities. So they could be important determinants of antioxidant power of plant originated foods. Besides promoting the sensory properties and colour of plant parts, they have important anti-microbial, anti-allergenic, anti-atherogenic, vasodilatory and cardioprotective functions [11]. Flavonoids, the strongest antioxidant class of naturally occurring phenolics, are ubiquitous in the plant kingdom. Many researchers have reported that flavonoids show bewildering functions in plants as metal chelators, radical scavengers and chain-breaking antioxidants [12]. Flavonoids may affect seed quality by altering digestibility and have some beneficial effects including lowering the risk of cancer types, heart diseases and the other age-related health problems [13].

The researches on the antioxidant capacity of peanut are generally focused only on the by-products such as skin and hull [14, 15, 16] but studies on peanut seeds with skin are scarce. The aim of the current work was to evaluate and compare some important nutritional and antioxidant properties of peanut seeds grown in Turkey.

2. Material and Method

Six certified varieties were used for the analysis. Peanut genotypes (Gazipaşa, Sultan, DA35/2011, NC-

7, Cihangir, Halisbey) were grown at the experimental field areas of Eastern Mediterranean Agricultural Research Institute at Doğankent, Adana, in 2016. Soon after collection, seeds were stored at 4°C. The unshelled seeds of the genotypes were ground into flour for analyzing the total antioxidant potential, the total phenolic and flavonoid levels, percentages of crude oil and protein amounts and fatty acid composition. Skins of seeds were not removed before grinding.

Crude oil amount of seeds was determined according to James [17] with some modifications. Crude oils were extracted from 5 g of ground samples using Soxhlet apparatus in 140 mL petroleum ether as a solvent. The extraction was carried out for 150 minutes. Petroleum ether was then removed from extracts at 105 °C for 1 hour. Extracts were cooled to room temperature at 30 minutes and weighted. Oil determination was performed through an automatic unit, Soxtherm Gerhardt Variostat (Germany). Results were expressed as percentages. Data was calculated using the equation: % Crude Oil Content = (Extracted oil weight X 100) / sample weight.

Crude oils were extracted in petroleum ether were used to analyse the fatty acid composition. Total lipids were methylated to obtain fatty acid methylesters [18]. A gas chromatograph (Agilent GC 7890A) was used to separate the methylesters. The samples (1 µL) were injected in triplicates. Sample peaks were compared with the peaks of standard methyl esters. Oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), palmitic acid (C16:0) and stearic acid (C18:0), were analysed in four replicates for each peanut genotype.

Kjeldahl procedure is the most widespread method for protein determination. The method is based on the estimation of the nitrogen content. In this study, crude protein amount of seeds was evaluated using Kjeldahl method of AOAC 990.03 [19]. Gerhardt-Turbotherm (Germany), Gerhardt-Vapodest (Germany) and Schott Titroline-Easy (Germany) devices were used for digestion, distillation and titration, respectively. 1 g of ground seeds was used for protein determination. Commercial Kjeldahl catalyst (including K₂SO₄, CuSO₄ and Se) and 12 ml of 98% H₂SO₄ were added to the samples to digest the organic materials. After combustion for 1 hour at 410 °C, samples were cooled. 0.1 N H₂SO₄ was used for titration until the colour change. Crude protein content was calculated as percentages using Kjeldahl nitrogen concentrations.

Phenolics and flavonoids were extracted from 0.5 g of ground seed in 80% methanol. 20 minutes-sonicated extracts were centrifuged at 14000 rpm for 5 minutes. Folin-Ciocalteu method was employed for determining the total phenolic amount of peanut flour [20]. 9 ml of distilled deionized water (dd H₂O)

was added on 1 mL of extract. 1 mL of Folin-Ciocalteu's phenol reagent was allowed to react with the solution. 10 mL of 7% Na₂CO₃ was then mixed with the previous solution at the 6th minute. In order to reach the final volume (25 mL), dd H₂O was added to the flask. After 90 minutes, the absorbance was detected spectrophotometrically at 750 nm (UV-mini 1240-Shimadzu). A standard curve was prepared via repeating the same procedure to the gallic acid solutions (20-100 mg/L).

Total flavonoid amounts of peanut flour were determined utilizing the aluminium chloride colorimetric test [21]. 3 mL dd H₂O was added to 1 mL of flour extract. 0.3 mL of 5% NaNO₂ was blended with the solution. 0.3 mL of 10% AlCl₃ was put into the mixture. 2 mL of 1 M NaOH was then allowed to react with the solution. The final volume was completed to 10 mL with dd H₂O. The absorbance was read at 510 nm spectrophotometrically. The same steps were carried out to catechin solutions (20-100 mg/L) to draw a standard curve.

Antioxidant activity was based on ABTS method, which was adapted from Thaipong et al. [8]. 3 g of seed flour was homogenized ultrasonically in 25 mL of methanol. After storing at 4 °C for 12 hours, the homogenates were centrifuged for 20 minutes at 15000 rpm. Supernatants were taken for analysis. 7.4 mM ABTS⁺ and 2.6 mM potassium persulfate were mixed in equal volumes and kept for 12 hours at 25 °C. Dark condition was ensured during the storage of the mixture. 1 mL solution was diluted by adding 45 mL of methanol to get an absorbance of 1.17±0.02 at 734 nm. 2850 µl ABTS⁺ solution was blended with 150 µL of extracts at various concentrations (25, 250, 500 and 1000 µg mL⁻¹). Test tubes were stored for 2 hours in dark conditions. The absorbances were read at 734 nm. A standard curve was obtained from the solutions of 25-600 µM Trolox. ABTS⁺ radical scavenging activity was calculated utilizing the equation (% Inhibition = [(A_{control reaction}-A_{sample reaction})/A_{control reaction}] X 100) mentioned by Gaafar et al. [10] before.

Data attained from the work was statistically analysed via SPSS Statistics 23 software. Results at P<0.05 were judged to be significant.

3. Results

The crude oil contents of seeds varied from 43.9 to 45.9% (Figure 1). Oil percentages of Gazipaşa, Sultan,

DA35/2011, NC-7, Cihangir, Halisbey were 45.0, 43.9, 44.7, 46.4, 45.5 and 45.9, respectively. Statistically, no significant difference was observed among the samples with respect to crude oil amounts.

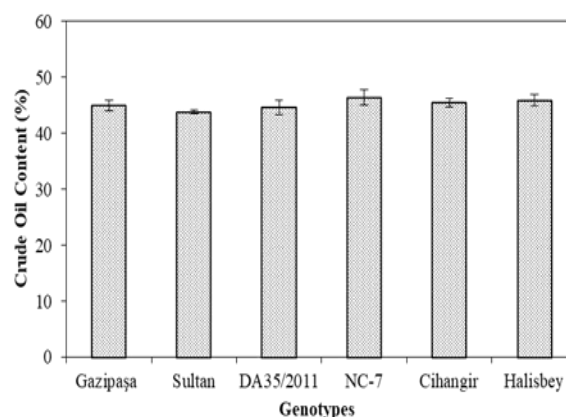


Figure 1. Crude oil content (%) of peanut genotypes

The fatty acid composition of six peanut genotypes was showed in Table 1. In agreement with previous researches, our data indicated that oleic acid was the most prevalent fatty acid in all genotypes. Oleic acid contents ranged from 48.99% to 60.81% among the genotypes and the highest oleic acid amounts were obtained from NC-7 (60.81%) and DA35/2011 (59.69%). The second most abundant fatty acid, linoleic acid, varied between 20.22% and 29.96%. NC-7 (20.22%) and DA35/2011 (20.98%) had the lowest linoleic acid contents. Thus, these two genotypes were noticeable with their highest oleic acid/linoleic acid ratio. On the contrary, Cihangir had the lowest oleic acid (48.99%)/linoleic acid (29.96%) ratio. Palmitic acid made up 8.73-10.53% of the all fatty acids. Gazipaşa (10.53%) and Cihangir (10.46%) were the richest genotypes in palmitic acid contents while palmitic acid levels of NC-7 (8.73%) and DA35/2011 (8.85%) were significantly lower than the other seed types. Significant differences were found within stearic acid values of peanut genotypes. Gazipaşa (2.84%), Cihangir (3.13%) and Halisbey (3.10%) exhibited lower stearic acid contents than DA35/2011 (3.50%), Sultan (3.49%) and NC-7 (3.36%). However, linolenic acid, a polyunsaturated fatty acid, was found in trace amounts in all genotypes. Linolenic acid values of the samples ranged from 0.97% to 1.07%. Sultan (0.97%) was the poorest genotype with regard to linolenic acid values. Halisbey (1.07%) and Cihangir (1.03%) contained the highest linolenic acid contents notably compared with those of the other genotypes.

Table 1. Fatty acid compositions (% of total oil content) of peanut genotypes

Genotypes	Fatty acids (%)				
	Palmitic acid	Stearic aci	Oleic acid	Linoleic acid	Linolenic acid
Gazipaşa	10.53±0.10	2.84±0.04	50.54±0.33	28.66±0.69	1.02±0.01
Sultan	10.00±0.09	3.49±0.10	53.18±0.30	25.99±0.32	0.97±0.01
DA35/2011	8.85±0.07	3.50±0.06	59.69±0.63	20.98±0.54	1.00±0.01
NC-7	8.73±0.10	3.36±0.04	60.81±0.35	20.22±0.25	1.00±0.01
Cihangir	10.46±0.05	3.13±0.19	48.99±0.15	29.96±0.15	1.03±0.03
Halisbey	9.76±0.10	3.10±0.13	52.42±0.51	26.82±0.42	1.07±0.02

The crude protein contents varied between 26.9-30.6%. NC-7 showed the highest protein content (Figure 2). DA35/2011 (29.6%) was the second protein-rich seed type whereas the protein content of this genotype was still significantly higher than other four genotypes. There was no noticeable difference in the values acquired from the genotypes Sultan (26.9%) and Cihangir (27.2%). Similarly, there was no significant difference between the crude protein levels of Gazipaşa (27.7%) and Halisbey (27.8%).

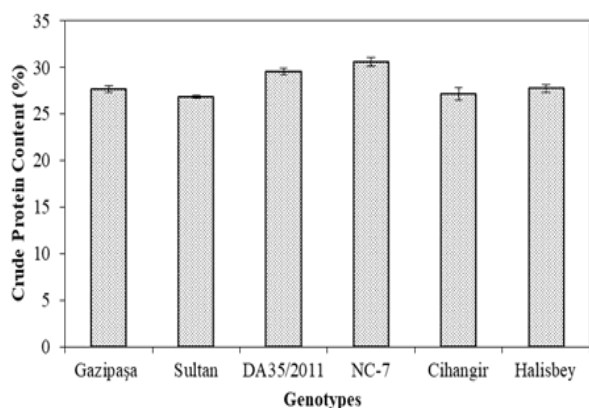


Figure 2. Crude protein content (%) of peanut genotypes

Raw peanut seeds with skins were milled to determine the phenolic and flavonoid amounts along with the antioxidant activity. The total phenolic content of the genotypes extended from 34.9 (DA35/2011) to 187.9 (Gazipaşa) mg GAE 100 g seed⁻¹ (Figure 3). Each result was significantly different from the others. The total phenolic contents of the other genotypes were 128.9 (Sultan), 121.8 (Cihangir), 108.0 (Halisbey) and 36.2 (NC-7) mg GAE 100 g seed⁻¹.

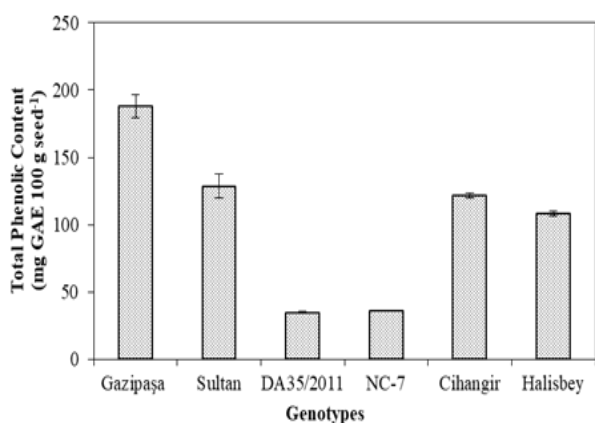


Figure 3. Total phenolic content of peanut genotypes

The total flavonoid content of the genotypes changed from 29.5 (NC-7) to 111.2 (Gazipaşa) mg CE 100 g seed⁻¹ (Figure 4). No significant difference was observed between DA35/2011 (29.8 mg CE 100 g seed⁻¹) and NC-7. Also, the results obtained from Sultan (58.7 mg CE 100 g seed⁻¹) and Cihangir (63.1 mg CE 100 g seed⁻¹) were statistically similar.

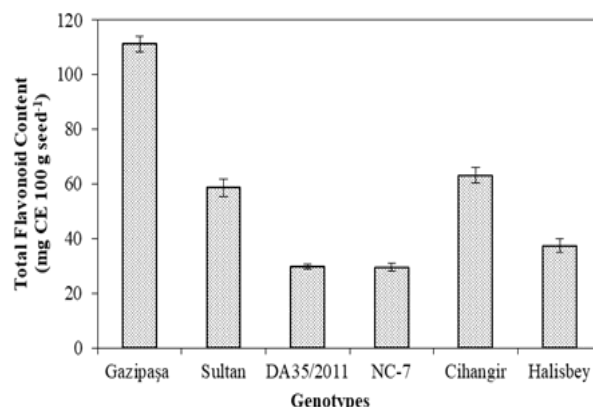


Figure 4. Total flavonoid content of peanut genotypes

In this study, a widespread decolourization method was used for screening the radical scavenging activity. Different dilutions of seed methanolic extracts were used to calculate the inhibition percentages (Table 2). According to the results, at a concentration of 1000 µg/ml, Gazipaşa and Sultan displayed the highest total antioxidant activities (31.61% and 30.42%) while the least antioxidant capacities were observed in DA35/2011 (22.86%) and NC-7 (23.70%).

4. Discussion and Conclusion

Peanut, as a multipurpose oilseed, is the fifth major source of plant oil worldwide [22]. The present study is pointed out that the crude oil contents of our seeds were in acceptable ranges that mentioned previously by Gulluoglu et al. [4] and Ayoola et al. [23]. The crude oil amounts of our genotypes showed very close values to each other. It is already known that the nutritional benefit of peanut is mainly based on its unsaturated fat and cholesterol-free oil [24]. Thus, apart from its prevalent usage as a snack in our country, we can clearly conclude that all six genotypes are suitable for oil and peanut butter production.

Fatty acid composition of seeds is crucial for the taste and quality of peanut and peanut products [25] and

Table 2. ABTS⁺ radical scavenging activity of peanut seed extracts

Genotypes	Inhibition %			
	25 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Gazipaşa	12.69±1.15	18.62±0.30	22.22±1.43	31.61±1.24
Sultan	16.49±0.13	21.73±0.23	25.78±0.47	30.42±0.86
DA35/2011	8.79±0.13	13.93±0.23	15.60±0.47	22.86±0.86
NC-7	10.47±0.13	12.69±0.77	19.01±0.26	23.70±0.23
Cihangir	13.73±0.47	16.79±0.86	20.84±0.97	28.79±1.96
Halisbey	11.65±0.13	19.16±0.23	23.36±0.47	26.86±0.86

pretty much dependent on the cultivar. Our genotypes exhibited different profiles according to the fatty acid contents. Peanut breeders focused on the increased oleic acid levels for prolonged oil stability [26]. However, it has been proved that an enhanced shelf life and a decreased rancidity could result from not only an elevated oleic acid amount but also a high oleic acid/linoleic acid ratio [27]. The present study showed that the predominant fatty acid was oleic acid in all seeds while NC-7 and DA35/2011 exhibited the highest oleic acid/linoleic acid ratios. Oleic, linoleic and palmitic acid amounts comprised about 90% of the fatty acid composition of the peanut oil whereas the other fatty acids could not exceed 5%, individually [27, 28]. Moreover, Maguire et al. [6] reported that the saturated fatty acid composition of peanut seeds mainly consisted of palmitic acid and stearic acid. Results of the present study confirmed that the most abundant saturated fatty acid for peanut seeds was palmitic acid. Dwivedi et al. [29] noted that there was a negative relationship between palmitic and oleic acid contents while the correlation between palmitic and linoleic acid contents was positive. The results of our study were consistent with those reported by previous works [29, 30]. Our data showed that selected genotypes may be acceptable as rich sources of unsaturated fatty acids likewise previously published studies [6]. NC-7 and DA35/2011 came into prominence with their higher oleic/linoleic acid ratio than the other genotypes.

The nutritional benefit of peanut seeds is arisen from not only the unsaturated oil content, but also their plant-originated protein. Peanut is a legume plant and contains more protein than all other nuts [31]. Our crude protein results were in accordance with the previous reports [32, 33]. All genotypes showed excellent protein amounts with slight differences. However, NC-7 was the most potent seed as a vegetable protein source.

Limmongkon et al. [34] pointed out that peanut seeds are rich in human health-associated phytochemicals such as plant phenolics. According to the results of this study, the total phenolic and the total flavonoid contents exhibited remarkable differences considering the genotypes. The amounts of these metabolites are known to be affected by cultivars and/or growth conditions [32]. Due to the fact that growth conditions of all genotypes were standardized in the present study, different values should be resulted from genotypes. Talcott et al. [35] suggested that normal oleic varieties contain approximately 50% oleic acid while high oleic peanut varieties contain exceed 80% oleic acid. Our genotypes may be accepted as normal oleic peanut varieties. And normal oleic peanut varieties have the highest polyphenolic compounds [32]. So the oleic acid content and phenolic levels of peanut seeds have effects on each other. Peanut seed phenolic levels have been confirmed to retard fat rancidity [16].

Therefore, the phenolic content level is one of the crucial features to estimate the peanut oil and butter quality. On the contrary to their relatively high oleic acid content, NC-7 and DA35/2011 had the least total phenolic compounds. Thus, our results confirm the assumption of Talcott et al. [35]. On the other hand, Gazipaşa was the most promising genotype according to its total phenolic content.

Flavonoids are one of the most abundant phenolic compounds in peanut seeds [36]. Our study showed that the total phenolic levels of all seed types were compatible with the total flavonoid amounts. The most flavonoid accumulation was detected in Gazipaşa and the poorest seeds were NC-7 and DA35/2011. Pratt and Miller [37] reported that the flavonoids extracted from peanut kernels exhibited antioxidant activity. Because flavonoids may directly scavenge $O_2^{\cdot-}$ and $\cdot OH$ by single electron transfer [38].

The antioxidant potential of peanut seeds was verified using the ABTS assay. The discoloration of the ABTS⁺ radical medium presented the existence of the antioxidants in our samples. Gazipaşa, the richest genotype in the total phenolic and the total flavonoid contents, showed the strongest radical scavenging activity. NC-7 and DA35/2011 had the lowest level of antioxidant potential, likewise the lowest total phenolic and the total flavonoid contents. Our results confirmed the previous studies showed that the antioxidant powers of the genotypes were well-matched with both the plant phenolics and flavonoids [39, 40].

The results of this work revealed that the nutritional and antioxidative components of peanut seeds influenced by genotype. The highest phenolic content meant the highest antioxidant capacity. Nevertheless, no direct relation was observed between the nutritional characteristics and antioxidant power. With their high antioxidant capacities, the seeds of Gazipaşa and Sultan are suggested for functional food production.

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