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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Chemical Composition, Antioxidant Capacity And Total Phenolic Content of Hazelnuts Grown In Different Countries

Farklı Ülkelerde Yetiştirilmiş Fındıkların Antioksidan Kapasitesi, Fenolik İçeriği ve Kimyasal Kompozisyonu

Sümeyye SAHIN[1]*, Tahsin TONKAZ[2], Tarık YARILGAÇ[3]

Abstract

Hazelnut providing the macro-and micronutrients is a constituent of the healthy diet. Hazelnut, one of the most consumed treenut, is produced in the different countries. The geographical origin influences the chemical composition and the biological activity in the several plant foods. The purpose of this study is to evaluate the chemical composition, the antioxidative capacity and total phenolic content of the hazelnut kernels obtained from the different countries, including Azerbaijan, Chile, Italy, and Turkey. The hazelnut kernels were examined for crude oil, ash, moisture, and protein contents. The refractive index, iodine value, and fatty acid composition of the hazelnut oils were identified. In addition, the hazelnut oil, the kernels, and the defatted kernels were tested for their antioxidative activities and total phenolic contents. Protein contents of the hazelnuts from different countries were similar. The largest oil yield was determined in the hazelnut kernels from Chile and Turkey (62.35 ± 0.51 % and 62.29 ± 0.46 %, respectively). While the oil from Turkish hazelnut kernels showed the highest oleic acid content (84.09 ± 0.17 %), Azerbaijan hazelnut oil had the lowest oleic acid content (78.10 ± 0.48). The highest phenolic content was detected in the hazelnut kernels from Azerbaijan. Although the lowest phenolic content was observed in the hazelnut kernels from Turkey, the most potent antioxidative capacity was determined in the Turkish hazelnut kernels, their oil, and their defatted form. It can be concluded that the Turkish hazelnut kernels could contain high amounts of the fat-soluble antioxidants in addition to the water-soluble phenolic antioxidants. The results suggested that the hazelnuts exhibited different chemical composition, antioxidant capacity, and phenolic content depending on their origin.

Keywords: Hazelnut oil, Geographical origin, Fatty acid composition, Antioxidant capacity, Total phenolic content

[1]***Sorumlu Yazar/Corresponding Author:** Sümeyye ŞAHİN, Department of Food Engineering, Ordu University, 52200 Ordu, Turkey. E-mail: sumeyyesahin@odu.edu.tr OrcID: 0000-0002-9344-7690

[2] Tahsin TONKAZ, Department of Biosystem Engineering, Ordu University, 52200 Ordu, Turkey. E-mail: ttonkaz@odu.edu.tr OrcID: 0000-0002-7136-1562

[3] Tarık YARILGAÇ, Department of Horticulture, Ordu University, 52200 Ordu, Turkey. E-mail: t.yarilgac@odu.edu.tr OrcID: 0000-0003-2097-7161.

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Öz

Makro- ve mikro besin elementlerini karşılayan fındık, sağlıklı beslenmenin bir parçasıdır. En çok tüketilen ağaç yemişlerinden biri olan fındık farklı ülkelerde yetiştirilmektedir. Bilindiği üzere çeşitli bitkisel gıdalarda, coğrafi orjin bitkinin kimyasal bileşimi ve biyolojik aktivitesini etkilemektedir. Bu çalışmanın amacı, Azerbaycan, Şili, İtalya ve Türkiye olmak üzere 4 farklı ülkeden temin edilmiş fındık numunelerinin kimyasal kompozisyonu, antioksidan kapasitesi ve toplam fenolik içeriğini değerlendirmektir. Çalışma kapsamında fındık numunelerinin ham yağ, kül, nem ve protein içerikleri analiz edilmiştir. Bu fındık numunelerinden elde edilen yağların refraktif indeks, iyot sayısı ve yağ asidi kompozisyonları belirlenmiştir. Buna ilaveten fındık yağı, yağı alınmış fındık ve işlem yapılmamış fındık numunelerinin antioksidan içerikleri ile toplam fenolik içerikleri analiz edilmiştir. Çalışma bulgularına göre farklı ülkelere ait fındık numunelerinin protein içeriklerinin benzer olduğu saptanmıştır. Calışmada en fazla yağ verimi Şili ve Türkiye'ye ait fındık numunelerinde belirlenmiştir (sırasıyla % 62.35 ± 0.51 ve % 62.29 ± 0.46). Türkiye'den temin edilen fındıklardan elde edilen yağların en yüksek oleik asit içeriğine (% 84.09 ± 0.17) sahip iken, Azerbaycan fındık numuneleri yağlarının en düşük oleik asit içeriğine (% 78.10 ± 0.48) sahip olduğu bulunmuştur. En yüksek toplam fenolik içeriğine Azerbaycan'dan temin edilen fındık numunelerinde saptanmıştır. En düşük toplam fenolik içeriğinin Türkiye'ye ait fındık numunelerinde gözlemlenmesine rağmen, en yüksek antioksidan kapasitesinin de yine bu Türkiye'den elde edilen fındık numunelerinin, bunlardan elde edilen yağ ve yağsız numunelerin sahip olduğu bulunmuştur. Bu durum Türkiye'ye ait fındık numunelerinin suda çözünebilen fenolik antioksidanlara ek olarak yüksek miktarda yağda çözünebilen antioksidan da içerebileceğini göstermektedir. Çalışma sonuçları, fındığın orjinine bağlı olarak farklı kimyasal bileşim, antioksidan kapasitesi ve fenolik içeriğe sahip olduğunu ortaya koymuştur.

Anahtar Kelimeler: Fındık yağı, Coğrafik orjin, Yağ asiti kompozisyonu, Antioksidan kapasite, Toplam fenolik içerik

1. Introduction

According to WHO, a healthy diet containing nuts helps to prevent malnutrition as well as a range of noncommunicable diseases (WHO, 2019). Hazelnut (*Corylus avellana* L.) is one of the most popular edible tree nuts and the second most produced tree nuts in the world after almonds (Alasalvar and Shahidi, 2009). The total production of hazelnut with shell in the world is 1006178 tones and Turkey, the main hazelnut-producing country, produces 675000 tones (67 % of total production) in 2017. Italy is the second largest hazelnut producer, accounting for roughly 13 percent of the world's production, followed by Azerbaijan with 4.3 percent of production. Although Chile is a new hazelnut producer, it provides 1.8 percent of the global supply (FAO, 2019).

Hazelnuts, rich in macronutrients and micronutrients, are widely consumed as whole nuts. Among the constituents, fat is the major component, ranging from 43.8 to 69.0 % (Amaral et al., 2006; Çetin et al., 2020; Köksal et al., 2006; Li and Parry, 2011; Savage et al., 1997). The hazelnut and its oils are also used for cooking, baking, in salad dressings, and in cosmetic products (Alasalvar and Shahidi, 2009; Güner et al., 2017). Hazelnut oil is characterized by high oleic acid content, a monounsaturated fatty acids (MUFA). Since hazelnut oils have small amounts of saturated fatty acids and high percentage of unsaturated fatty acids (Alasalvar et al., 2006; Amaral et al., 2006; Çetin et al., 2020; Crews et al., 2005; Ghirardello et al., 2013; Köksal et al., 2006; Li and Parry, 2011; Maguire et al., 2004; Savage et al.,1997), they provide health benefits, such as decreasing the risk of developing heart disease (Alasalvar and Shahidi, 2009; WHO, 2019). In addition to fats, hazelnuts are a good source of quality proteins which contain essential amino acids, particularly arginine, leucine, phenylalanine and valine. Protein content of hazelnuts varies from 9.3 to 22.5 g/100 g (Amaral et al., 2006; Çetin et al., 2020; Köksal et al., 2006; Savage and McNeil, 1998; Venkatachalam and Sathe, 2006).

Hazelnut is rich in water-soluble and fat-soluble vitamins. It serves a great source of vitamin E, which is a lipid soluble, powerful antioxidant and has health benefits acids (Alasalvar et al., 2006; Crews et al., 2005; Köksal et al.,2006; Savage et al., 1997). In addition, hazelnut contains phytochemicals described as calori-free, natural bioactive compounds such as phenolic compounds (Alasalvar and Shahidi, 2009). Several studies indicated total phenolic content and also phenolic compounds (Solar and Stampar, 2011; Güner et al., 2017) identified as phenolic acids acting as antioxidant (Altun et al., 2011; Arcan and Yemenicioğlu, 2009; Contini et al., 2008; Li and Parry, 2011). Recently, the total antioxidant capacity and antioxidant active compounds in hazelnut have been reported (Altun et al., 2011; Arcan and Yemenicioğlu, 2009; Contini et al., 2008; Delgado et al., 2010; Ghirardello et al., 2013; Li and Parry, 2011; Miraliakbari and Shahidi, 2008; Shahidi et al., 2007; Yang et al., 2009).

Although the nutritional and chemical composition and also the total antioxidant capacity and phenolic contents in different Turkish hazelnut cultivars have been demonstrated, the differences in chemical composition and biological activity of hazelnuts from different countries are not fully evaluated. The aim of the present study was, therefore, to investigate the chemical composition, antioxidant capacity and phenolic content of hazelnut kernels obtained from different countries, including three countries producing the most nuts in the world (Turkey, Italy, Azerbaijan) and a new hazelnut producer, Chile.

2. Materials and Methods

2.1. Materials

The dry hazelnuts without shells were obtained from marketplaces and supermarkets in Italy, Chile, Turkey, and Azerbaijan. The hazelnut kernels were grounded in a coffee mill prior to analysis.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ethyl alcohol, sodium thiosulfate, diethyl ether, DPPH (2,2-diphenyl-1-picrylhydrazyl), 25% potassium methoxide solution in methanol, gallic acid, CCl_4, potassium iodide, and Na_2CO_3, were purchased from Sigma-Aldrich (St. Louis, USA). Hexane, Wijs, folin-ciocalteu, n-Butanol, and 25% sulfuric acid were obtained from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Determination of proximate composition

The moisture content of hazelnut samples was determined using oven drying at 105 °C. To determine ash content, 3 grams of each hazelnut sample were kept in the ceramic crucibles at 550 °C until a constant final weight for ash was

achieved. Total fat was extracted 2 hours with n-hexane by using Soxhalet extraction apparatus (Velp Ser 148, Milano, Italy). Kjeldahl method was used for determination of protein content, which was calculated as total N x 6.25 (James, 1995.

2.2.2. Determination of fatty acid composition

Fatty acid composition of the hazelnut oils was determined by gas chromatograph (GC) according to the method described by Petersen et al. (2012). The oils were derivatized to fatty acid methyl esters (FAME; Restek, US) using 25% potassium methoxide solution in methanol. After neutralization with 25% sulfuric acid, FAMEs were extracted and injected into a TR-CN100 column (60 m × 0.25 mm I.D., 0.20 µm film thickness; Teknokroma, Spain) for seperation. The column was connected to a Shimadzu 2010 Series (Shimadzu, Tokyo, Japan) GC with a flame ionization detector (FID). The injector temperature was 140°C. Nitrogen was used as the carrier gas (30 mL/min). The temperatures of the oven, and detector were 250°C. FAMEs were identified in the hazelnut oils by comparing the retention times of the unknown peaks with those of the standard FAMEs.

2.2.3. Determination of refractive index and iodine value

To measure the refractive index of hazelnut oil, an Abbe refractometer was used. Analysis of iodine value was performed by the Wijs method according to literature (Shimamoto et al., 2016) with some modifications. The hazelnut oil samples (0.20 g) were dissolved using 15 mL of solvent (CCl_4) and 25 mL of Wijs solution. After incubation for 1 h in the dark, this solution was added to 20 mL of the potassium iodide solution (100 g/L) and 150 mL of distilled water. Then, 2 or 3 drops of 1 percent starch solution was added. Titration with 0.1 N sodium thiosulfate solution was carried out until the blue color disappeared.

2.2.4. Determination of total phenolic content

Total phenol contents of hazelnut kernel and defatted hazelnut kernel extracts were assayed by the Folin-Ciocalteu method described by Kaplan et al. (2019) slightly modified. 20 µL of kernel extract were mixed with 1300 µL distilled water and 50 µL Folin-Ciocalteu reagents. After incubation at room temperature for 2 min, 50 µL of Na2CO3 (7.5%, w/v) were added to the mixture. Then, the mixture was allowed to stand at room temperature for 60 min. The absorbance of the mixture was measured at 765 nm with a Perkin–Elmer Lambda 35 UV/Vis spectrophotometer (Perkin–Elmer 710 Bridgeport Avenue Shelton, CT 06484-4794, USA). Gallic acid was chosen as a standard. Total phenol contents in hazelnut extracts were calculated using standard curves. Results were expressed as millimoles per liter gallic acid equivalents.

2.2.5. Determination of total antioxidative activity

To determine the antioxidant capacity of the hazelnut kernels, defatted hazelnut kernels, and hazelnut oils, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed by the method according to literature (Sahin, 2011). 40 µL of the samples were added to 1.5 mL of DPPH solution (0.6 mM in n-butanol) in a microcuvette. After the mixture was incubated at room temperature for 30 min, the absorbance of the mixture was determined spectrophotochemically at 515 nm. Trolox was the standard used to calculate antioxidant capacity.

2.2.6. Statistical analysis

Data were statistically analysed using the Holm-Sidak multiple comparisons test following repeated measures two-way ANOVA ($p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$) by the software GraphPad Prism 6 (GraphPad Software, La Jolla, USA).

3. Result and Discussion

The moisture, ash, protein and oil content of hazelnut kernels are shown in *Table 1*. The moisture and ash contents were 2.80- 5.31 % and 2.34-3.79 %, respectively. Similar results for moisture and ash of hazelnut grown in Turkey have been shown before (Alasalvar et al., 2003; Çetin et al., 2020; Gunes et al., 2010; Köksal et al., 2006; Özdemir and Akinci, 2004; Seyhan et al., 2007). Hazelnuts from Italy yielded the highest amount of protein (19.15 %) whereas hazelnuts grown in Azerbaijan had the lowest protein yield (16.48 %). The protein contents of hazelnut samples grown in Turkey were similar to those reported by Çetin et al. (2020), and by Köksal et al. (2006). Hazelnuts of Chile and Turkey showed highest oil content (62.35% and 62.29%, respectively), followed by Italy (54.34%) and Azerbaijan (53.82%). The oil yield of hazelnut grown in Turkey was generally in good agreement with the results reported by

Çetin et al. (2020), by Kıralan et al. (2015), by Köksal et al. (2006), and by Li and Parry (2011). On the other hand, the oil content of Italian hazelnuts found in this study was lower than that determined by Ghiradello et al. (2013). Several factors, including growing conditions, harvest time, fruit maturity, geographic origin, seed diversity, clime, environmental conditions etc. can affect the amount of fat (Alasalvar et al., 2006; Köksal et al., 2006; Li and Parry 2011). To our knowledge, moisture, ash, protein, and oil content of hazelnut kernels grown in Azerbaijan and Chile have not been described before.

Table 1. Chemical composition of hazelnut kernels

	Moisture (%)	Total ash (%)	Protein (%)	Total oil (%)
Azerbaijan	2.80 ± 0.63	2.71 ± 0.19	16.48 ± 0.43	53.82 ± 0.28
Chile	4.15 ± 0.63	2.57 ± 0.05	18.76 ± 0.09	62.35 ± 0.51
Italy	5.04 ± 0.37	3.79 ± 0.09	19.15 ± 0.12	54.34 ± 0.44
Turkey	5.31 ± 0.04	2.34 ± 0.13	18.69 ± 0.09	62.29 ± 0.46

Table 2 gives fatty acid composition, iodine value, and refractive index of hazelnut oils. Hazelnut oil is naturally high in oleic acid (C 18:1 n 9; monounsaturated fatty acid) and its oleic acid content varies from 73.8 % to 85.7 % (Alasalvar et al., 2006; Crews et al., 2005; Çetin et al., 2020; Ghirardello et al., 2013; Köksal et al., 2006; Li and Parry, 2011; Maguire et al., 2004; Savage et al.,1997). In this study, the most abundant fatty acid was oleic acid, in the range from 78.1 % in hazelnut from Azerbaijan to 84.09 % in hazelnut from Turkey. Linoleic acid (C 18:2 n 9, 12; polyunsaturated fatty acid) ranged from 7.11 (hazelnut of Turkey) to 9.69 % (hazelnut of Italy). Similar results of oleic acid and linoleic acid have been reported for hazelnut from Turkey (Alasalvar et al., 2006; Crews et al., 2005; Çetin et al., 2020; Köksal et al., 2006; Li and Parry, 2011) and Italy (Crews et al., 2005; Ghirardello et al., 2013). It has been also reported that fatty acid contents in hazelnut varies depending on geographical location and varieties (Crews et al., 2005; Köksal et al., 2006; Li and Parry, 2011). Significant differences were also observed in the iodine value which is a measure of the degree of unsaturation in fatty acids. The lowest iodine value was determined in hazelnut oil from Turkey ($p<0.0001$), whereas hazelnut oil from Chile had the highest value ($p<0.0001$). Iodine values of hazelnut oils were generally in good agreement with the results published by Crews et al. (2005). It was found that there were no significant differences between refractive indexes of hazelnut oil samples ($p>0.05$) and refractive indexes (1.466-1.468) were similar to those reported by Firestone (2013).

Table 2. Chemical composition and quality parameters of hazelnut oil samples

	Fatty Acid Composition (%)				Iodine value	Refractive Index (25 °C)
	C 16:0	C 18:0	C 18:1 c 9	C 18:2 c 9, c 12		
Azerbaijan	8.04 ± 0.19	3.27 ± 0.08	78.10 ± 0.48	7.92 ± 0.10	86.38 ± 0.44	1.466 ± 0.00
Chile	6.97 ± 0.02	2.87 ± 0.02	80.22 ± 0.03	9.67 ± 0.03	90.12 ± 0.14	1.468 ± 0.00
Italy	5.96 ± 0.02	2.86 ± 0.05	81.31 ± 0.02	9.69 ± 0.02	88.86 ± 0.23	1.467 ± 0.00
Turkey	5.45 ± 0.1	3.21 ± 0.04	84.09 ± 0.17	7.11 ± 0.02	81.43 ± 0.42	1.467 ± 0.00

It was shown previously that hazelnut has high antioxidative activity analysed by several methods such as TAC, ORAC, CUPRAC, TOSC, ABTS/ persulfate, AAPH-linoleic acid assay, and DPPH (Altun et al., 2011; Arcan and Yemenicioğlu, 2009; Contini et al., 2008; Delgado et al., 2010; Ghirardello et al., 2013; Li and Parry, 2011; Miraliakbari and Shahidi, 2008; Shahidi et al., 2007; Yang et al., 2009). In this study, we evaluated the antioxidant capacity of hazelnut kernels, hazelnut oils and defatted hazelnut kernels using the DPPH-radical assay *(Figure 1).* Among the hazelnut oil samples, the oil of hazelnuts grown in Turkey exhibited the highest antioxidative capacity (1.48 mmol/L trolox equivalents), followed by hazelnut oil from Azerbaijan and Italy (1.06 and 0.84 mmol/L trolox equivalents, respectively), and finally from Chile (0.30 mmol/L trolox equivalents). The trolox equivalence value of hazelnut oil from Turkey was 4.9-fold higher than that of oil of hazelnut grown in Chile. Among the defatted hazelnut kernels, the highest DPPH radical scavenging capacity was also observed in the Turkish defatted hazelnut kernels (1.66 mmol/L trolox equivalents), followed by Azerbaijan and Italy (0.77 and 0.59 mmol/L trolox equivalents), and finally Chile (0.42 mmol/L trolox equivalents). From these results it can be decuded that the hazelnuts grown in Turkey are

rich in not only fat-soluble antioxidants, but also water-soluble antioxidants. Among fat-soluble antioxidants, Alasalvar et al. (2006) and Köksal et al. (2006) detected and quantified a high amount of α-tocopherol, the other tocopherols and small amounts of tocotrienols in oil from Turkish hazelnut (Alasalvar et al., 2006; Köksal et al., 2006). Altun et al. (2011) identified some phenolic compounds as water soluble antioxidants in Turkish hazelnut. When tested the hazelnut kernels, the Turkish hazelnut kernels demonstrated highest antioxidant capacity, followed by, in descending order, Azerbaijan > Italy > Chile *(Figure 1).* These results are in agreement with those reported by Li and Paryy (2011) showing that the antioxidant capacity of hazelnut is influenced by the geographical origin of hazelnut.

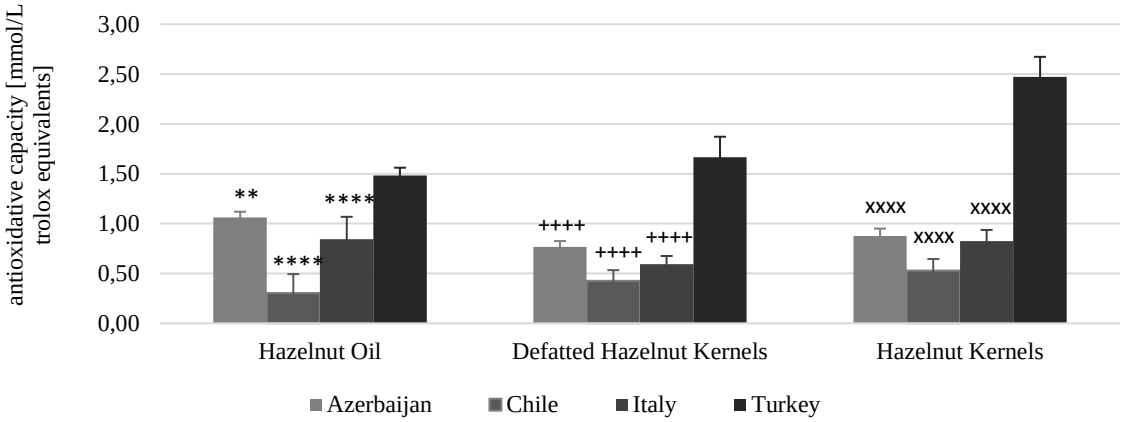

Figure 1. Antioxidant capacity of hazelnut oil, hazelnut kernel and defatted hazelnut kernel. The data are reported as mean ± standard deviation; n=3. **p < 0.01, and ****p < 0.0001 compared to hazelnut oil from Turkey; ++++ p < 0.0001 compared to defatted hazelnut kernels from Turkey; xxxx p < 0.0001 compared to hazelnut kernels from Turkey.

In addition, we evaluated the total phenolic content of hazelnut kernels and defatted hazelnut kernels by the Folin-Ciocalteu assay. The results are given in *Figure 2*. The highest phenolic content was determined in defatted hazelnut kernels from Azerbaijan (0.47 mmol/L), while kernels of hazelnut grown in Turkey had the lowest phenolic content (0.21 mmol/L). Interestingly, despite its low phenolic content, Turkish hazelnut exhibited the highest antioxidative activity *(Figure 1).* Therefore, it can be concluded that beside phenolic compounds, hazelnut contains some components having high antioxidant activity. Similarly, it has been shown that although the total amounts of phenolic compounds in Turkish hazelnut were lower than those in hazelnut from North America (Oregon), Turkish hazelnut had greater antioxidant capacity (Li and Parry, 2011). In contrast, Arcan and Yemenicioğlu (2009) reported a positive correlation between the total phenolic content and antioxidant activity in some hazelnut samples. It can be concluded that relationship between antioxidant capacity and total phenolic in hazelnuts may vary depending on their origin.

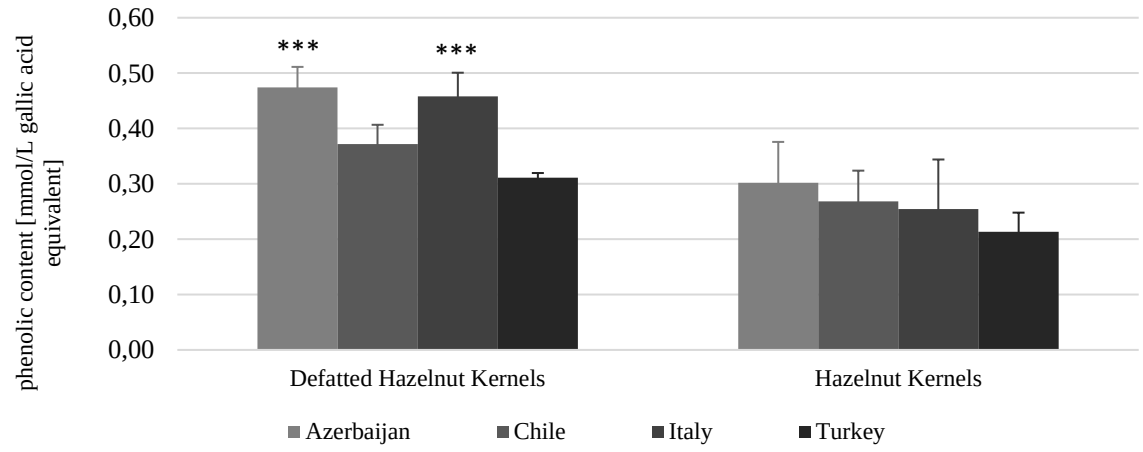

Figure 2. Total phenolic content hazelnut kernel and defatted hazelnut kernel. The data are reported as mean ± standard deviation; n=3. ***p < 0.001 compared to defatted hazelnut kernels from Turkey.

Conclusion

A comprehensive investigation of chemical composition, total phenolic contents and antioxidant capacity of hazelnut from 4 different countries was performed for the first time in this study. The amount of ash, protein content, oil yield, fatty acid composition and iodine value in this study clearly indicated that the geographical origin affects the chemical composition of hazelnut kernels. The whole hazelnut kernels, defatted hazelnut kernels and oil of hazelnut kernels were separately tested for total phenolic contents and antioxidant activity. Total antioxidant activity and phenolic contents varied also depending on growing conditions of hazelnuts. Further experiments are required to determine the antioxidative active compounds which are responsible for differences between the antioxidant activities of hazelnut samples from different countries.

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