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Araştırma Makalesi / Research Article

# Chemical Profile and Antioxidant Activity of Giresun Quality Hazelnut Skin

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## Abstract

Hazelnut skin is the thin and brown outer tissue layer, completely surrounds the hazelnut kernel and is released as a food by-product during the roasting process. Beyond its diverse composition including dietary fiber, phenolic compounds, flavonoids, vitamins, and minerals, hazelnut husk demonstrates substantial antioxidant activity. The chemical composition and the nutritional value of hazelnut and hazelnut skin can vary according to hazelnut varieties and the region where they grow. In this study, the chemical profile and antioxidant activity of skins from hazelnut grown in Giresun in the Black Sea Region of Türkiye were determined and its potential for use as a functional food component was investigated. Hazelnut skin samples extracted with different solvents such as acetone/methanol and ethanol/methanol, were found to have total phenolic compounds equivalent to 437.45 GAEq (mg GA/g sample) and 307.38 GAEq (mg GA/g sample), respectively. The fat content of the hazelnut skin was found to be 35.1% by mass on the dry matter, and the ratio of dietary fiber was found to be 53.5%. In addition, the total antioxidant value was calculated in samples extracted with different solvents that was found at 3.38 ± 0.2 mmol TE/g – 2.67 ± 0.1 mmol TE/g, respectively. Functional cake enriched with hazelnut skin was made, and sensory analysis studies of this functional product were also carried out.

**Keywords:** Hazelnut skin, Giresun, Antioxidant activity, Total phenolics.

# Giresun Kalite Fındık Zarının Kimyasal Profili ve Antioksidan Aktivitesi

## Öz

Fındık zarı, fındığı tamamen saran ince ve kahverengi dış doku tabakasıdır ve kavurma işlemi sırasında gıda yan ürünü olarak açığa çıkar. Genellikle kavurma işleminden sonra fındıktan ayrılır ve fındık endüstrisindeki yan ürünlerden biridir. Fındık zarı diyet lifi, fenolik bileşenler, flavonoidler, vitamin ve mineral gibi birçok faydalı bileşen içerir ve yüksek antioksidan kapasiteye sahiptir. Fındık ve fındık zarının kimyasal bileşimi ve besin değeri fındık çeşidine ve yetiştirildiği bölgeye göre değişiklik gösterebilmektedir. Bu çalışmada, Türkiye'nin Karadeniz Bölgesi'nde Giresun'da yetiştirilen fındıklardan yan ürün olarak elde edilen fındık zarının kimyasal profili ve antioksidan aktivitesi belirlenerek fonksiyonel gıda bileşeni olarak kullanım potansiyeli araştırılmıştır. Aseton/metanol ve etanol/metanol ile ekstrakte edilen fındık zarı örneklerinde sırasıyla 437.45 GAEq (mg GA/g örnek) ve 307.38 GAEq (mg GA/g örnek) eşdeğerinde toplam fenolik madde olduğu bulunmuştur. Fındık zarına ait toplam yağ miktarı kuru madde üzerinden kütlece %35,1 iken, diyet lifi oranı %53,5 olarak bulunmuştur. Ayrıca, Farklı çözücülerle ekstrakte edilen fındık zarı örneklerinde toplam antioksidan değeri 3.38 ± 0.2 mmol TE/g ve 2.67 ± 0.1 mmol TE/g fındık zarı eşdeğeri olarak hesaplanmıştır. Fındık Zarı ile zenginleştirilmiş fonksiyonel kek üretimi yapılmış, ayrıca bu fonksiyonel ürünün duyusal analiz çalışmaları yapılmıştır.

**Anahtar Kelimeler:** Fındık zarı, Giresun, Antioksidan aktivite, Total fenolikler.

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## 1. Introduction

Hazelnuts (*Corylus avellana* L.) have the largest share among Türkiye export products and cover almost all of the agricultural areas of the Black Sea Region of Türkiye (TMO, 2012). Hazelnuts and their components such as hazelnut oil, dietary fibers, and hazelnut skin have a good and essential source of human nutrition. Hazelnut skin is the thin and brown outer tissue layer that completely surrounds the hazelnut kernel and it is separated from the hazelnut after roasting process and a form one of the by-products in the hazelnut industry. They are mostly arising as waste materials or used as animal feed by most companies (Uzuner and Cekmecelioğlu, 2014; Del Rio et al., 2011).

The predominant constituent of hazelnut skin is dietary fiber (Tuncgil, 2020), with additional presence of various beneficial nutrients such as phenolic compounds and flavonoids, accompanied by a notable antioxidant capacity. In addition to these properties, hazelnut skin offers the potential to be used as a natural coloring additive and provides a natural brown color to foods (Özdemir et al., 2014; Balasundram et al., 2002).

The chemical composition of hazelnuts and other by-products such as hazelnut skin, hard shell, green leafy cover and tree leaf vary depending on the region where they were grown. In the literature, some studies have been conducted on hazelnut and by-products from different hazelnut varieties and different regions (Pfeil et al., 2024; Pelvan et al., 2018; Yuan et al., 2018; Xu et al., 2012; Locatelli et al. 2010). Even Though, eighteen different hazelnut varieties cultivated in Türkiye, Giresun Tombul hazelnuts is a type of grown in Giresun province, is grading as high-quality (premium quality) (Islam, 2018). It has been previously reported that the hazelnuts have a rich source of polyphenolic components, unsaturated fatty acids, essential amino acids, vitamins and minerals, and also have important flavonoids and high antioxidant activity exhibiting components (Alasalvar et al., 2003; 2009a; 2009b)

The current study examines the chemical composition and constituents of hazelnut skins (HS) sourced as a by-product from Giresun quality hazelnuts cultivated in the Black Sea Region of Türkiye, and explores their potential utility as functional ingredients in diverse food formulations.

## 2. Materials and Methods

### 2.1. Chemicals and Materials

Acetone, ethanol, methanol, and n-hexane were of reagent-grade level and purchased from Sigma–Aldrich. All chemicals gallic acid, Folin–Ciocalteu, potassium persulfate, ABTS reagent [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] were supplied by Sigma Aldrich (Steinheim,

Germany). Trolox [6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid], Sodium Carbonate, Acetic Acid, Sulfuric Acid and citric acid monohydrate were purchased from Merck (Germany). Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Milford, MA, USA).

Hazelnut skin samples (Tombul variety) used in the study were kindly provided from Fiskobirlik Ltd. (Giresun province, Türkiye), at the harvest season of 2020. They were obtained from Giresun Quality hazelnut kernels (*Corylus avellana* L.), cultivated in areas of Giresun province. Brown skin of hazelnuts was separated after roasting at 150 °C for 30 min from dried unshelled hazelnuts and ground using a domestic type grinder (Delonghi KG49 grinder). The samples were stored in the dark at -20 $^{o}$C until analyzed. Ground hazelnut skin was chemically characterized for its basic composition.

### 2.2. Proximate Composition Analysis of Hazelnut Skin

Moisture content was determined by using Standard AACC (American Association of Cereal Chemists) methods (AACC, 2000). Ground hazelnut skin was used for moisture determination. The device's special weighing cup is placed on the device (Ohaus Mb35). Approximately 5 g of the ground sample is placed in a container and distributed homogeneously. Drying was achieved at 110 (±2) °C by selecting the standard program.

Water activity ($a_w$) was determined by using Water Activity Meter (Aqua Lab Dew Point Water Activity Meter 4). The ground sample is evenly spread within the sample chamber to form a mass of uniform thickness that completely covers the base. A sample is then placed in the device calibrated with a NaCl/$H_2O$ saturated solution containing salt crystals, and measurements are conducted at 20°C. Each measurement takes approximately 10-15 minutes, during which equilibrium is achieved.

Color measurement of hazelnut skin was determined by using the Hunter method (Hunterlab Miniscan Ez 4500l). For the analysis, ground hazelnut skin is placed inside a petri dish in a manner that covers the entire surface of the dish. Subsequently, the petri dish is placed on a white background, and readings are taken using the Hunter device. The color of ground hazelnut skin was investigated via the L* (lightness), a* (redness) and b* (yellowness) and ΔE (color difference) parameters.

Total lipid contents were determined by extracting the crude oil with hexane (Merck, Darmstadt, Germany) from dried hazelnut samples using a Soxhlet extraction apparatus (Şimşek Laborteknik PI 400, Türkiye). 5 g of the sample is weighed onto a coarse filter paper, which is then folded and placed into a cartridge, with cotton placed on top. The cartridge is then inserted into the Soxhlet extraction unit at 130 °C for 6 hours. Defatted samples were used for protein and ash contents determinations.

Total protein contents were determined according to the Kjeldahl Method using an automatic distillation unit (UDK-149; VELP). Total protein content was calculated by multiplying the total nitrogen content by a factor of 6.25 (N × 6.25) according to Association of Official Analytical Chemists methods (AOAC, 1990). Ash contents were determined by weight difference after combustion of the samples at 550 °C in a furnace (Protherm furnaces, Ankara, Turkey) for 14 h (Tuncil & Celik, 2019). All proximate analyses were triplicate.

### 2.3. Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu method, widely favored for determining total phenolic content, relies on measuring absorbance based on the color intensity formed through the Folin reactive agent (Huang et al., 2005). This method operates on the principle of phenolic compounds, which are soluble in water and other organic solvents, forming colored complexes with the Folin reagent in an alkaline environment (Slinkard and Singleton, 1977). Its extensive application stems from its methodological ease, reproducibility, and strong correlation with alternative techniques. The results of this method are typically expressed in terms of a standard phenolic compound, often equated to gallic acid and catechin.

Total phenolic content (TPC) of hazelnut skin was determined using sequential extraction procedure (SEP) in order to evaluate the solvent effects of extraction process. In the sequential extraction procedure, hazelnut skin samples were first treated with acetone or ethanol solvents, and then the solid part was extracted again with methanol as triplicate.

Total phenol determination was carried out using the Folin-Ciocalteau colorimetric method. In the analysis, 0.5 mL of the sample was mixed with 2.5 mL of freshly prepared 1N Folin solution and left in the dark for 2.5 minutes. Then, 7% $Na_2CO_3$ solution was added, and the mixture was left to react in the dark for 1 hour. The color intensity resulting from phenolic components was determined by measuring absorbance values at 725 nm using a UV-Visible Spectrophotometer (Mapada Uv-6100psc). The total phenol value was expressed in terms of gallic acid equivalents (GAE) using a calibration curve constructed with gallic acid.

### 2.4. Determination of The Total Antioxidant Activity

Antioxidant activity of hazelnut skin was determined using ABTS method. Hazelnut skin was extracted with acetone/methanol and ethanol/methanol using sequential extraction procedure. Trolox equivalent antioxidant capacity (TEAC) determination is a method based on the radical scavenging effects of antioxidant components present in the extract. In this method, the ABTS+ radical formed

by oxidation with 7 mM ABTS/2.45 mM $K_2S_8O_2$ reaction gives a color that can be determined spectrophotometrically at 734 nm (Huang et al., 2005; Re et al., 1999; Serpen et al. 2008). The initial absorbance value is adjusted to 0.70 (±0.03), and the color loss caused by the extract added to the solution at appropriate dilutions (at a concentration that can inhibit the blank absorbance by 20-80%) is monitored kinetically (Mapada Uv-6100psc). Through a calibration curve generated with Trolox (0–600 µg/mL), antioxidant activity determination is achieved as mmol Trolox equivalent (TE) per gram of sample.

### 2.5. Dietary Fiber Analyses

Dietary fiber analyses were conducted using the Total dietary fiber Kit (Merck). The working principle of the Total Dietary Fiber analysis kit was based on the combination of enzymatic and gravimetric methods. Since the sample contained more than 5% fat, the fat in the sample was removed. The sample was shaken three times with 25 mL of petroleum ether and then filtered. Subsequently, the obtained fat-free sample was dried at 70°C for 2-3 hours. The fat-free sample was ground to a particle size of 0.3-0.5 mm mesh.

The analysis was performed through the following stages:

A. Enzymatic hydrolysis

- 1g of the sample was weighed into 50 mL Falcon tubes. Then, 40 mL of MES/TRIS solution was added onto the sample, and the solution is vortexed.
- 50 µl of α-amylase solution was added, vortexed, and incubated at 95-100°C for 30 minutes. The solution was then cooled to 60°C.
- 50 µl of protease solution was added, vortexed, and incubated at 60°C for 30 minutes.
- 5 mL of 0.561 N HCl was added to adjust the solution's pH to the range of 4.1-4.8.
- 150 µl of amyl glucosidase solution was added, vortexed, and incubated at 60°C for 30 minutes.

B. Total dietary fiber analysis

- After the enzymatic digestion process was completed, the sample was transferred to a beaker containing 220 mL of ethanol at 60°C.
- The solution was left to stand for 1 hour with the lid closed
- It was then filtered through previously tared Gooch crucibles under vacuum.
- The precipitate was washed with 78% ethanol (3x15 mL), 95% ethanol (2x10 mL), and acetone (3x10 mL).
- It was left in an oven at 105°C overnight, Weight is recorded.

### 2.6. Functional Cake Production Enriched with Hazelnut Skin

Functional cake enriched with hazelnut skin was made as homemade style. The recipe used for the production of functional cake is given in Table 1. While preparing cakes enriched with hazelnut skin, 4 different samples were prepared together with the control sample by changing the hazelnut skin ratio. All used materials quantities and applied conditions as the heat treatments were kept at the same in these samples. The only changed parameter between products was the amount of hazelnut skin. In this way, the sensory analysis was applied to see the effects of hazelnut skin on the product. The ingredients in the recipe were prepared by weighing and mixed with a mixer (Tefal, Masterblend Pro Activ Flow Technology). 30 g portions were taken from the mixture and hazelnut skin were added into each mixture as 7%, 10%, 13% by weight. The mixtures were poured into cake molds and baked in the oven (Luxell, granitech) initially heated at 160°C for a 30 min.

**Table 1.** Production recipe for cake

| Ingredients | Quantity |
|---|---|
| Sugar | 250 g |
| Eggs | 3 pieces (L size) |
| Milk | 200 mL |
| oil | 160 mL |
| Baking powder | 10 g |
| Vanilla powder | 5 g |
| White Flour | 200 g |

### 2.7. Sampling Procedure and Direct Measurement of The Antioxidant Capacity in Functional Products

Functional nutritive properties in cakes formulated to include hazelnut skin were monitored by comparing the antioxidant capacities acquired directly from the samples. The homogeneous distribution of hazelnut skin within the products is very important. In this process, sampling had to be done meticulously, so a representative sample was obtained using cluster sampling. The sample with reduced particle size was divided into 4 different clusters in equal weights, and samples were taken from all these clusters and brought together. This process was repeated to create four new clusters from the combined sample, and samples were again taken from each cluster, pooled, and utilized for measurements. All measurements were conducted in triplicate to ensure reliability and consistency. The decolorization of the solution containing 1.5 mL of $ABTS^{+}$ reagent adjusted to an

absorbance of 1.797, upon the addition of 20 ± 1.5 mg of cake or cookie samples, was monitored. Measurements were intermittently taken from the mixture, which was centrifuged at 3000 rpm for 3 minutes, with absorbance recorded at 734 nm, and the reaction continued accordingly. Monitoring lasted for 4.5 hours in cake samples with a negative control also included in the observation.

### 2.8. Statistical analysis

All experiments were performed in triplicate, all quantitative data are expressed as mean values of at least three analytical determinations with the standard deviation.

## 3. Results and discussion

### 3.1. Proximate composition of hazelnut skin

In this study, chemical analyses such as total phenolic content analysis (TPC), total antioxidant activity analysis, color determination, water activity tests, moisture, protein, total dietary fiber and total oil content were conducted to determine the chemical and physical properties of Giresun quality hazelnut skin.

Table 2 shows proximate composition of roasted hazelnut skin. Protein (7.10%), moisture (4.8%), water activity (aw) (0.3517) and ash (1.8%) contents of hazelnut skin were found a proximate composition similar to those reported by Locatelli et al. (2010), Anil (2007), and Ozdemir et al., 2014. Roasted hazelnut skin color was determined using the parameters, L* (lightness), a* (redness) and b* (yellowness) and also ΔE (color difference). The L*, a*and b* values were found 32.20 ±0.66, 11.29 ± 0.12 and 16.57 ± 0.15, respectively. Hazelnut skin exhibited brown color intensity. This shows that it can be used as a functional ingredient when brown coloring agent is needed.

Total dietary fiber was found to be the main component of hazelnut skin with a contribution of 53.5%. High dietary fiber amount has an importance in terms of health benefits; therefore, although hazelnut skin are waste materials of hazelnut industry, they can be considered as a functional food ingredient in food formulations.

Total lipid content of hazelnut skin was 35.1 ± 1.2% which almost 2-times higher than that reported by others (Anil, 2007; Özdemir et al. 2014). These differences could be attributed from differences in the cultivated land of hazelnuts. Additionally, when different techniques or roasting processes are used to remove the hazelnut skins, it is known that the skin absorbs the oil during the process (Alasalvar et al., 2009, Pelvan et al., 2018), as well as the amount of oil absorbed by the skin could possibly was impacted by different roasting procedures.

**Table 2.** Chemical composition of hazelnut skin

| Component | Amount | | |
|---|---|---|---|
| Moisture | 4.8 ± 0.04% | | |
| Total dietary fibers | 53.5 ± 1.2% | | |
| Proteins | 7.10± 0.3% | | |
| Oil (% Mass on Dry Matter) | 35.1 ± 1.2% | | |
| Ash | 1.7 ± 0.1% | | |
| Total phenolic content | 437.5 ± 7 mg GAE/g -307.4 GAEq | | |
| Total antioxidant capacity | 3.38 ± 0.2 mmol TE/g – 2.67 ± 0.1 mmol TE/g | | |
| Color measurement values (Average ± SD) | L* (lightness) | a* (redness) | b* (yellowness) |
| | 32.20 ±0.66 | 11.29 ± 0.12 | 16.57 ± 0.15 |
| Water activity (aw) | 0.3517 | | |

Results are expressed as mean ± SD (n = 3).

Total phenolic content (TPC) of roasted hazelnut skin in acetone/methanol and ethanol/methanol extract (See Figure 1) were found to 437.5 mg GAE/g and 307.4 GAEq (mg GA / g sample), respectively. Monagas et al. (2009) and Ozdemir et al. (2014) was studied with different cultivated hazelnut skin and reported total phenolic content of 107 mg GAE/g and 233.7 mg GAE/g, respectively. This difference might be because of the various hazelnut component, extraction conditions and extraction solvents. However, total phenolic content of hazelnut skin was found to be higher compared with the other nut skins such as almond (22.8 mg GAE/g), peanut (73.9 mg GAE/g), walnut (101 mg GAE/g) and pistachio (116.32 mg GAE/g) (Monagas et al., 2009; Salcedo et al., 2010; Tomaino et al., 2010) (See Table 1, Figure 1).

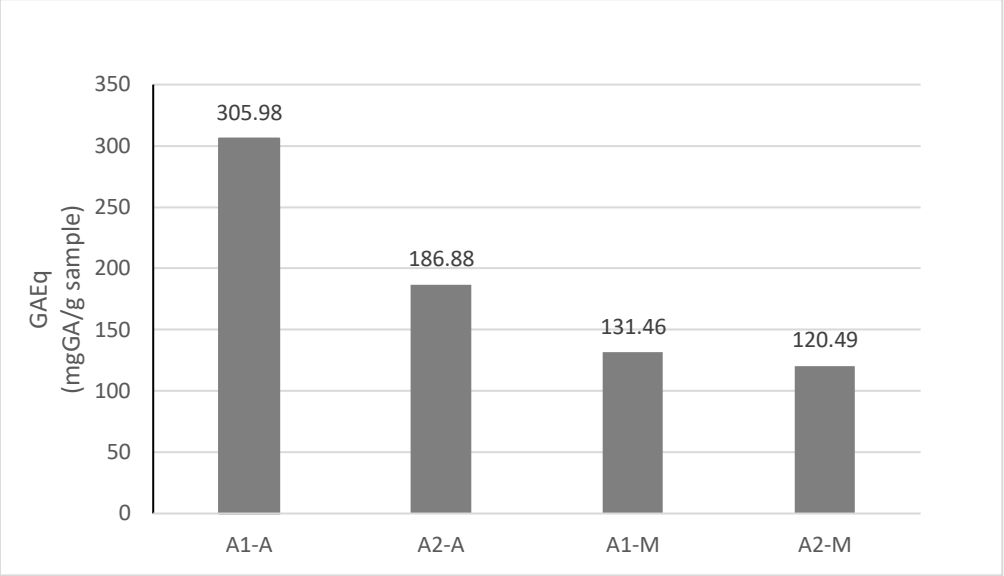


**Figure 1.** Total Phenoliccontent of hazelnut skins in different extraction solvents A1-A: Extraction with acetone, A2-E: Extraction with ethanol, A1-M: Extraction with methanol after acetone, A2-M: Extraction with methanol after ethanol

Total antioxidant of hazelnut skin was determined in acetone/methanol and ethanol/methanol extract that was found at 3.38 ± 0.2 mmol TE/g – 2.67 ± 0.1 mmol TE/g, respectively (Table 1, Figure 2). The highest antioxidant value in the obtained extracts was achieved in the extraction with acetone + methanol solvents. Total antioxidant capacity of hazelnut skin was found higher to those reported by Shahidi et al., 2007; and Ozdemir et al. 2014. However, total antioxidant capacity of hazelnut skin was higher than those of other nut skins such as almond, peanut, walnut and pistachio (Monagas et al., 2009; Salcedo et al., 2010; Tomaino et al., 2010).

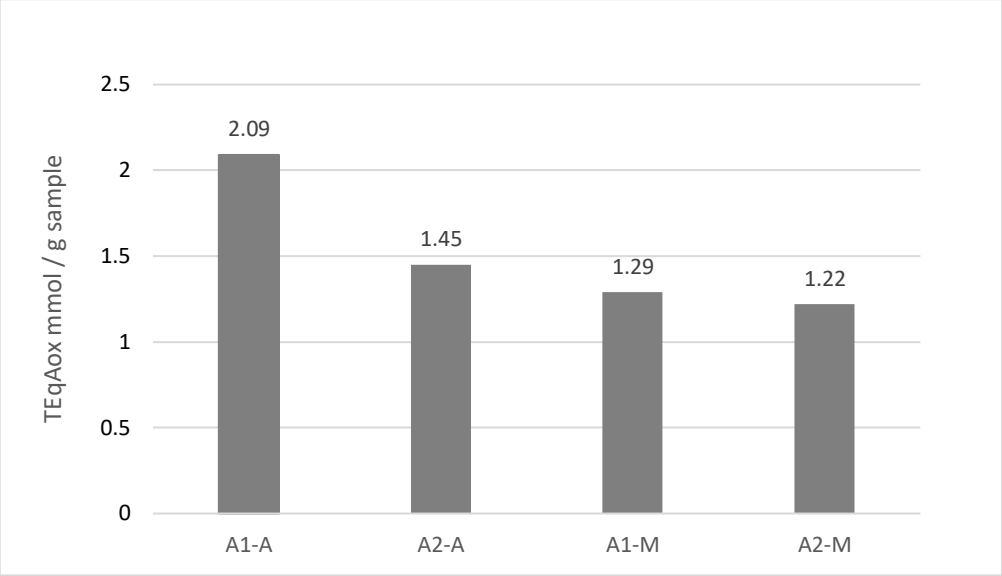


**Figure 2.** Antioxidant activity of hazelnut skins in different extraction solvents A1-A: Extraction with acetone, A2-E: Extraction with ethanol, A1-M: Extraction with methanol after acetone, A2-M: Extraction with methanol after ethanol

### 3.2. Functional Cake Enriched with Hazelnut Skin and Sensory analysis

Hazelnut skin (HS) were used as functional food ingredient to enrich the cake. By changing the hazelnut skin ratio (%7, %10 and %13), three (3) different functional cake samples were prepared as well as the control sample (see Figure 3).

The direct measure of the antioxidant capacity of functional cake enriched with hazelnut skin was made. Hazelnut skin addition led to a significant increase in the reduction rate of $ABTS^{+}$ reagent, as depicted in the figure 4. It was observed that the antioxidant capacity of the cakes increased by 3 to 8 times with the addition of HS, depending on the amount used. Notably, there was a strong correlation (0.982) between the quantity of added HS and the extent of decolorization.

In this way, the sensory analysis was applied on the functional product to see the hazelnut skin effects on flavor. The sensory analysis test conducted with 30 participants. The results show that the most popular and frequently preferred product was 7% hazelnut skin containing cake among the cake products with 7%, 10% and 13% hazelnut skin. Additionally, 60% of the participants stated that they liked the product and wanted to consume it.

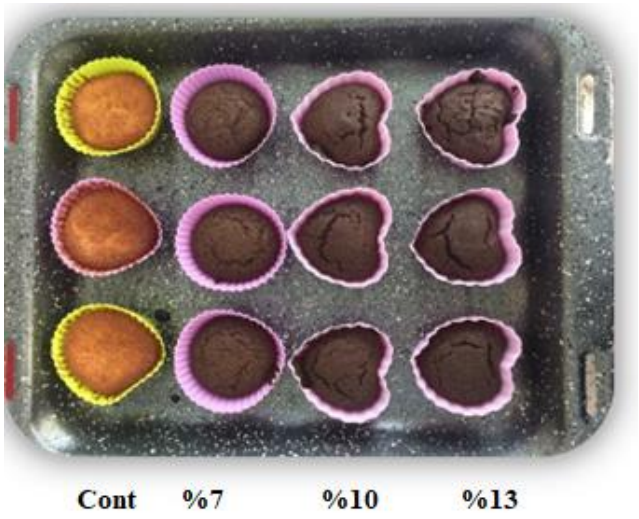


**Figure 3.** Cake Enriched with Hazelnut Skin,
Cont: Control samples, and %7, %10 and %13 Enriched with Hazelnut Skin

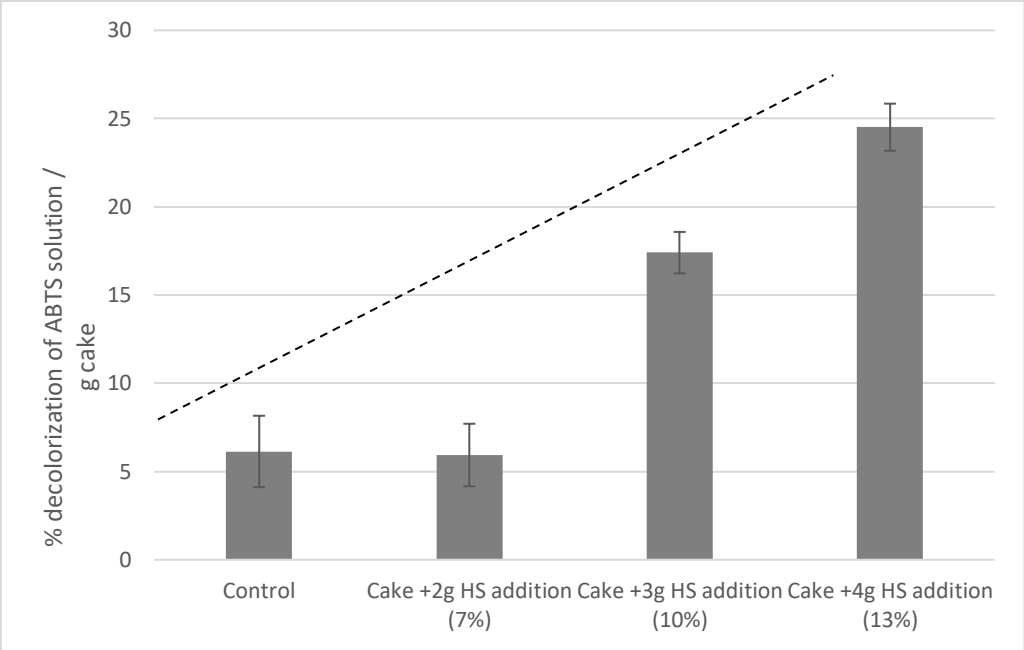


**Figure 4.** Effect of HS addition on the antioxidant capacity of the products.

## 4. Conclusions

In this study, the chemical composition of Giresun Quality hazelnut skins (HS) and usability as functional food ingredient was evaluated. Hazelnut skin (HS) was obtained as a by-product in the roasting process of Giresun Quality hazelnuts. Total phenolic compounds in acetone/methanol and ethanol/methanol extract was found at 437.45 GAEq (mg GA/ g sample) and 307.38 GAEq (mg GA / g sample), respectively. The fat content of the hazelnut skin was determined 35.1% by mass on the

dry matter and the dietary fiber was 53.5%. Total antioxidant was found at 3.38 ± 0.2 mmol TE/g in acetone/methanol and 2.67 ± 0.1 mmol TE/g as equivalent in ethanol/methanol extract. Additionally, Hazelnut skin (HS) was used as functional food ingredient to enrich the cake. Concluding, Giresun Quality hazelnut skins (HS) can be considered a low-cost natural source of antioxidants, dietary fiber and also colorant and it has a potential in application as functional ingredient of various food products.

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**Statement of Conflicts of Interest**

There is no conflict of interest between the authors.

**Statement of Research and Publication Ethics**

The authors declare that this study complies with Research and Publication Ethics.

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