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Research Article

BIOACCESSIBILITY OF POLYPHENOLS FROM HAZELNUT SKIN AND THEIR USE IN NOODLE FORMULATION

Bige İncedayı*, Nihal Türkmen Erol

Bursa Uludag University, Faculty of Agriculture, Department of Food Engineering, Bursa, Turkey

**Corresponding Author:*
E-mail: bige@uludag.edu.tr

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ABSTRACT. Hazelnut skin is a valuable waste as it contains a large amount of polyphenols, which have many health effects, including antioxidant and anticancer properties and the prevention of cardiovascular diseases. In this study, total polyphenol content, antioxidant capacity and in-vitro digestion of polyphenols of the hazelnut skin were investigated. Gallic and ellagic acids, which are important phenolic compounds of hazelnut skin, were determined by HPLC. In addition, the potential use of hazelnut skin extract in noodle production was investigated in order to gain functionality to noodle, which is a widely consumed product. The hazelnut skin contained 49.00 mg gallic acid equivalent/g dry matter total polyphenol and had 62848.57 mmol ascorbic acid equivalent/100g dry matter antioxidant capacity. Gallic acid content (20.39 mg/g dry matter) of hazelnut skin was found to be approximately 5.4 times higher than ellagic acid (3.80 mg/g dry matter). After the gastrointestinal track, the total polyphenol content was lower than the initial value as the antioxidant capacity of the skin extract. The addition of the skin extract (0.4%) to the noodle dough increased the total polyphenol and antioxidant capacity of the final product compared to control. As in the skin extract, it was observed that the stability of the polyphenols from the noodle sample was higher in gastric stage than intestinal one. The addition of hazelnut skin extract to the noodle dough increased the bioaccessibility of the noodle polyphenols. Therefore, this study showed that hazelnut skin, as an important source of polyphenols, may be useful for food enrichment.

Keywords: *waste, bioactive, in-vitro digestion, enrichment*

INTRODUCTION

In the world, an average of 200 million tons of vegetative waste are generated during production every year. Most of these wastes are released directly to nature without any processing or used as fuel, animal feed or fertilizer. In recent years, due to environmental factors and economic reasons, studies about valorization of these wastes have increased. Hazelnut fruit (*Corylus avellana* L.) is mostly used as an ingredient for processed foods such as bakery and confectionary or consumed as raw. Hazelnut processing generates by-products such as the hard outer shell (65.5% by weight) and the inner skin covering the hazelnut kernel as a thin layer (2.5% of kernel weight) [1–3]. Previous studies have shown that hazelnut skin is rich source of bioactive polyphenols [4, 5].

The use of extracts from plants or their by-products containing bioactive substances such as phenolic compounds in various foods as an alternative to artificial additives or for food enrichment is increasing gradually. According to Amado *et al.* [6], found that the extract from potato peel waste prevented oxidation in soybean oil due to its antioxidant activity. Rashidinejad *et al.* [7], reported that both the total polyphenols and the total antioxidant capacity of cheeses obtained by adding green tea catechins to whole milk increased. While an increase in phenolic content and antioxidant activity of

fresh pasta enriched with extract from artichoke waste was observed, the extract decreased yellowness and increased brownness but did not change the textural and cooking parameters [7].

In previous studies conducted on the use of hazelnut skin in food, the hazelnut skin was directly added to bakery products [9] and fresh pasta [10] after only its grounding. However, there is no report about enrichment of foods by adding the polyphenol-rich extract from the hazelnut skin to any food, including noodle.

Although phenolic compounds have strong antioxidant activity, their bioactivity depends on their degree of bioaccessibility [11]. The release of compounds from food and their solubility during digestion are called bioaccessibility, and a high rate of bioaccessibility is required for intestinal absorption of these compounds. Assessing the actual bioavailability of a phenolic compound in the human or animal body is difficult and costly. Instead, the *in-vitro* gastrointestinal digestion method is a simpler and faster method used to obtain information about the release of a phenolic compound from the foodstuff and its stability in gastrointestinal conditions. In some previous studies conducted on different materials such as pomegranate peel flour [12], cocoa powder [13], apple [14] and elderberry fruit [15], the bioaccessibility of phenolic compounds was determined according to the *in-vitro* gastrointestinal digestion method, but in the literature, there has been no study on the bioaccessibility of hazelnut skin polyphenols.

In this study; It was aimed to (1) determine the TP, phenolic profile and AC of the extract from hazelnut skin, and the bioaccessibility of polyphenols (2) use hazelnut skin extract for enrichment in noodle production and thus to evaluate hazelnut skin, which is an important waste.

MATERIALS AND METHODS

Materials

The skins of hazelnut (*Corylus avellana L.*) used in the study were supplied from a hazelnut processing plant (Fiskobirlik, Ordu, Turkey). They were stored in polyethylene bags at 4±2 ºC until used. Flour and eggs were obtained from the local market.

Polyphenol extraction

Polyphenols were extracted from ground powder skin and noodle with a certain particle size (150-300 μm) using distilled water at solid to solvent ratio of 1/39.70 (w/v) and temperature of 60 °C for 22 min. For highest yield of polyphenols, extraction conditions applied were optimized using response surface methodology (RSM). After extraction, the mixture was rapidly cooled under tap water, centrifuged at 10.000 rpm for 15 min and filtered through Whatman No.1 filter paper. The clear extract was stored at -20 °C until used for analyses of TP, phenolic profile by HPLC, AC and bioaccessibility analysis. Each extraction was carried out in triplicate.

Total polyphenols (TP)

TP of the samples was determined using the Folin-Ciocalteu reagent according to ISO 14502-1:2005 method [16]. In this method, 0.5 mL of extract or pure water (as a blank) was mixed with 2.5 mL of Folin-Ciocalteu reagent (10%, v/v). 2 mL of 7.5% sodium carbonate solution is added to the mixture after 5 min and shaken thoroughly.

The mixture was allowed to stand for 60 min and blue color formed was measured at 765 nm against blank using a spectrophotometer (Shimadzu UV-VIS 1208).

A calibration curve of gallic acid (5-50 µg/mL) was prepared and the results determined from regression equation of the calibration curve (R^2=0.99) were expressed as mg gallic acid equivalents per gramme of dry matter (DM).

Determination of phenolic profile of the skin extract

The identification and quantification of individual polyphenols in the skin extract were performed on a HPLC system including LC-20 AD Shimadzu pumps, a CTO-10 ASVP column oven and SPD-M20A photo diode array (PDA) detector, a Shimadzu DGU-20A5R degasser and SLC-10 A VP system controller. A computer-controlled system with LC solution software was employed for data analysis. The column used was a C18 reversed phase Nova Select (250 × 4.6 mm ID, 5µm) and was operated at 25 °C. UV spectra were recorded from 190-370 nm and peak areas were measured at 270 nm. The two mobile phases used for gradient HPLC elution were (A) 0.1% orthophosphoric acid in water (w/v) and (B) acetonitrile. The gradient elution profile was as follows: from 0 to 6 min, 7% B; from 6 to 82 min, 7-38% B; from 82 to 90 min, 38-60% B. The column was re-equilibrated with the initial conditions for 5 min before the next injection. The flow rate was 1.0 mL/min. The injection volume was 20 µL.

Chromatographic peaks in the samples were identified by comparing their retention times and UV spectra with those of their reference standards and by co-chromatography with added standards. Quantification was performed from the peak area of each component and its corresponding calibration curve.

Antioxidant capacity (AC)

AC was determined by the 2,2,diphenyl-2-picryl-hydrazyl (DPPH) method of Türkmen Erol *et al.* [17]. An aliquot of 1950 µL of 6 x10^{-5} M DPPH radical in methanol was added to a test tube with 50 µL of extract. Instead of extract, pure water was used as control. The reaction mixture was vortex-mixed and let to stand at room temperature in the dark for 60 min, then the decrease in absorbance at 517 nm was measured.

AC was calculated as percentage inhibition (AC, %) of the DPPH radical by the following equation:

$$AC(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} x100$$

$Abs_{control}$: Absorbance of the solution of DPPH without sample,

Abs_{sample} : Absorbance of the solution of DPPH with sample

Standard curve of reference antioxidant ascorbic acid (0-150 µg/mL) was assayed under identical conditions for affinity to scavenge DPPH. Antioxidant capacity of samples was converted to ascorbic acid equivalent (AAE) defined as mmol of ascorbic acid equivalents per 100 g of DM. Greater values of the AAE are related to greater antioxidant capacity of the sample.

In-vitro digestion (bioaccessibility)

In-vitro digestion method was applied according to Minekus *et al.* [18] to evaluate the bioaccessibility of phenolic compounds of the sample extracts. It was carried out in two stages as gastric and intestinal. The method was briefly applied as follows.

Gastric phase

10 mL of extract was mixed with 7.5 mL of gastric fluid, 1.6 mL of stock pepsin solution, 5 mL of 0.3 M $CaCl_2$, 0.2 mL of 1M HCl (to adjust the pH to 3), and 0.695 µL of water. The mixture was incubated for 2 h at 37 °C in a shaking water bath (Memmert WNB 22), then centrifuged at 12.000 rpm for 10 min (Sigma 3K30) and filtered from Whatman no:1.

Intestinal phase 10 mL of gastric fraction, 5.5 mL of intestinal fluid, 2.5 mL of pancreatin solution, 1.25 mL of 160 mM bile, 20 µL of 0.3 M $CaCl_2$, 0.075 mL of 1 M NaOH (to adjust pH to 7), and 0.655 mL of water were mixed. The mixture was incubated at 37°C in a shaking water bath for 2 h.

For both stages, the mixtures were centrifuged at 12.000 rpm for 10 min (Sigma 3K30) and filtered from Whatman no:1. The blank was prepared under the same conditions using the same chemicals except for the extract.

After each stage, the amount of TP was determined by spectrophotometer and the bioaccessibility (%) of TP was calculated as follows;

$$Bioaccessibility\ (\%) = (C_{digested} / C_{undigested}) \times 100$$

$C_{digested}$: Concentration in digested sample after gastric/intestinal stage (mg)

$C_{undigested}$: Concentration in undigested sample (mg)

Noodle production

Noodle production was carried out according to Collins and Pangloli [19] with some modification. After the skin extract was freeze-dried (at –50 ºC and under 0.1 mbar/0.75 mmHg vacuum), it was used at a rate of 0.4% in noodle production. Noodle without skin extract formed the control group. Dry ingredients (wheat flour and dry extract) were combined and mixed in a bowl. Then, water and egg were added and the mixture was kneaded for 5 min using a dough mixer (Siemens FQ.1) until the dough stiffened. After that, the dough was hand-kneaded for 5 more min, divided into equal portions (shaped into a ball), wrapped with cling film and allowed to rest for 20 min at room temperature. At the end of the period, the dough was thinned first with a roller and then with a dough thinning machine (Titania, Italy). Dough was rested for 20 min at room temperature in order to remove excess moisture and thus prevent sticking that may occur during cutting. Then, the dough was cut into strips of 0.4 x 3 cm in two stages with a noodle cutting machine. Fresh noodles were spread on trays and dried at room temperature until the moisture content of noodle decreased to 8-9%. Dry noodles were stored at the same temperature in polyethylene bags until analysis.

Fig. 1. *Dough mixer and thinning machine*

Statistical analysis

Experimental results were expressed as means ± standard deviation of triplicate measurements and analyzed by SPSS software (SPSS statistics 23, IBM.2015). Analysis of variance was performed by one-way ANOVA procedure. Means were compared by using Duncan multiple comparison test. Values of $p < 0.05$ were considered as significantly different.

RESULTS AND DISCUSSION

The effect of in-vitro digestion on TP and AC of hazelnut skin extract

TP and AC values of hazelnut skin extract at initial and after digestion are given in Table 1. A higher amount of TP (49.00 mg GAE/g DM) was detected when compared to some previous studies. For example, 9.18 mg GAE/g shell in hazelnut shell [5], 7.51-18.51 mg GAE/g dw (dry weight) in sunflower seed meal [20] and a maximum of 1.13 mg GAE/g dw TP in potato peel [6] were reported. On the other hand, Zeppa *et al.* [10] found a higher level of TP (102.19 -195.76 mg GAE/g dw) in hazelnut skins of different varieties than that detected in this study. This can be due to cultivar diversity and different extraction conditions. However, the AC value (62848.57 mmol AAE/ 100g DM) observed in this study could not be compared with the literature values of the skin extract determined in with the literature values because of the fact that the same antioxidant method used in the studies was applied with some different modifications and the results were expressed in different units. According to some previous studies on AC of plant by-products, including hazelnut skin; 309-1375 µmol Trolox equivalent-TE/g skin [21] and 854.47- 1004.98 µM TE/g dw [22] in hazelnut skin, 2077- 5214 µmol TE /kg fresh weight [23] in artichoke waste and 3.24 mmol TE/g extract [24] in chestnut bark were detected.

The TP and AC of the skin extract showed a similar trend after digestion. They both significantly decreased compared to their initial values ($p < 0.05$) (Table 1). The highest decrease was observed in the intestinal phase. This is associated with lower stability of polyphenols due to the alkaline environment during intestinal digestion [25]. The reduction of TP and thus AC after gastrointestinal digestion has also been demonstrated in the previous studies with different foods. Bouayed *et al.* [14] stated that the TP content of four different apple cultivars (with an average initial TP level of 44.42 mg/ 100 g fresh weight) decreased to 35.95 mg/100 g fresh weight after the gastric stage and to 21.84 mg/100 g fresh weight after the pancreatic stage. Similarly, TP and AC values of ten different walnut varieties decreased by an average of 74.1% and 77%, respectively, after *in- vitro* digestion compared to their initial values [26].

However, unlike these results, Wang, Amigo-Benavent *et al.* [11] reported that TP and AC of grape pomace did not change after the gastric digestion, but decreased after the intestinal digestion compared to their initial values. According to the results of a study conducted with pomegranate products and wastes, the initial TP and AC values showed different trends after *in-vitro* digestion, depending on the material and the extraction solvent used. With respect to TP, both a decrease and an increase were observed at the end of both stages, while a decrease at the end of the gastric stage and an increase at the end of the intestinal stage were observed regarding AC [25]. The differences in the results might be due to the differences in stability of the polyphenols of the materials used and *in-vitro* digestion conditions applied.

Table 1. *TP (mg GAE/g DM), bioaccessibility of TP (%) and AC (mmol AAE/100g DM) values of hazelnut skin*

Digestion stage	TP	Bioaccessibility of TP	AC
Initial	$49.00 \pm 0.35^{c*}$	100.00 ± 0.00^{c}	62848.57 ± 1965.80^{c}
Gastric	21.02 ± 0.82^{b}	42.89 ± 0.69^{b}	21449.68 ± 633.88^{b}
Intestinal	14.29 ± 0.31^{a}	29.17 ± 0.25^{a}	14807.42 ± 129.64^{a}

*The differences between means in lower case letters in the same column are significant ($p < 0.05$).

Because high AC was detected in the hazelnut skin, in parallel with the amount of TP, phenolic compounds of the extract were examined by HPLC and as a result of the analysis, only ellagic (3.80 ± 0.10 mg/g DM) and gallic (20.39 ± 0.52 mg/g DM) acids which are important phenolic acids were detected. Both are phenolic compounds with high antioxidant activity, and gallic acid is better absorbed in the body than other polyphenols [27]. Because the gallic acid in hazelnut skin is 5.37 times more than ellagic acid, it is thought to be used more by the body. On the other hand, ellagic acid, a derivative of gallic acid, has antimutagenic, antiviral, antibacterial, antioxidant and anticarcinogenic properties [28, 29]. Consistent with this study, Shahidi *et al.* [30] determined the gallic acid as the highest phenolic acid among the others in the hazelnut skin.

The effect of in-vitro digestion on TP and AC of enriched noodle

In order to obtain enriched noodle, hazelnut skin extract was freeze-dried and added to the noodle dough. According to the results of the analysis, the addition of the extract significantly increased (120.75%) the TP content of the enriched noodle compared to the control one, as expected (Table 2). While AC was not detected in the control noodle, it was determined at the level of 103.37 mmol AAE/100g DM in the enriched noodle. The results obtained from the previous studies in which noodle [31] and fresh noodle [7] were enriched with pomegranate peel and artichoke waste extracts, respectively, were in agreement with the results of this study. However, the researchers found a lesser increase compared to this study. This could be due to the differences in the stability of the polyphenols of pomegranate peel (72.21 mg GAE/g; [32]) and artichoke waste (0.77-1.45 mg GAE/g fresh weight; [23]) and noodle processing conditions (kneading, thinning and drying, *etc*.).

In-vitro digestion significantly affected the TP content and AC of the noodle samples ($p < 0.05$) (Table 2). The TP content of the control and enriched noodle samples showed

a significant decrease ($p < 0.05$) after digestion, but more at the intestinal stage, as in the TP of the skin extract. Already, it is stated that polyphenols are sensitive to environmental factors such as pH change, light and heat, and are easily degraded by digestive enzymes [15]. On the other hand, it was observed that the bioaccessibility of noodle polyphenols was higher than the hazelnut skin polyphenols. This could be due to binding of polyphenols to the proteins in the composition of the noodle, increasing their stability during digestion [33]. In addition, the bioaccessibility of the polyphenols of the enriched noodle was higher than that of the control one (Table 2). After *in-vitro* digestion, the AC of the enriched noodle also significantly decreased due to the decrease in its TP content ($p < 0.05$). So, it had no AC after the intestinal stage.

Table 2. *TP (mg GAE/g DM), bioaccessibility of TP (%) and AC (mmol AAE/100g DM) values of noodle*

		Digestion Stage		
	Noodle	Initial	Gastric	Intestinal
TP	Control	$0.53 \pm 0.03^{c*}$	0.29 ± 0.01^{b}	0.12 ± 0.01^{a}
	Enriched	1.17 ± 0.03^{b}	0.71 ± 0.01^{a}	0.65 ± 0.04^{a}
TP bioaccessibility	Control	100.00 ± 0.00^{c}	53.75 ± 1.81^{b}	22.27 ± 2.64^{a}
	Enriched	100.00 ± 0.00^{b}	60.49 ± 1.77^{a}	55.45 ± 3.65^{a}
AC	Control	0	0	0
	Enriched	103.37 ± 0.66^{c}	42.15 ± 1.87^{b}	0

*The differences between means in lower case letters in the same ~~column~~ row are significant ($p < 0.05$).

CONCLUSION

In this study, hazelnut skin, which is an important industrial waste, was evaluated in terms of TP, bioaccessibility of polyphenols, phenolic compounds and AC. Due to the high TP content of the hazelnut skin, the skin extract was freeze-dried for use in the noodle formulation. It was determined that the noodle enriched with the hazelnut skin extract contained higher amount of polyphenols compared to the control noodle, and therefore, the enriched noodle showed AC although no AC was observed in the control one. On the other hand, the amount of TP and AC of the skin and noodles were significantly reduced during *in-vitro* digestion, but more at the intestinal stage ($p < 0.05$). However, this decrease was less in noodles due to their protein content. The results obtained from this study showed that hazelnut skin can be used as a good source of polyphenols for food enrichment.

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