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Evaluation of the bioaccessibility of peanut skin polyphenols and their potential use for food enrichment

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Abstract: Polyphenols obtained from agricultural and industrial residues are also considered as remarkable sources of natural antioxidants to replace synthetic ingredients. In this study, the contents of total polyphenols (TP) and total flavonoids (TF), antioxidant capacity (AC) and *in-vitro* bioaccessibility of polyphenols (as gastric and intestinal stages) of the extract from peanut skin using water were investigated. Additionally, the potential use of peanut skin extract in noodle production was researched in order to add functionality to noodle, which is a widely consumed product. The results showed that 71.67 mg gallic acid equivalent (GAE)/g dry matter (DM) of TP, 123.11 mg rutin equivalent (RE)/g DM of TF and 66267.46 mmol ascorbic acid equivalent (AAE)/100g DM of AC were found in peanut skin. After the gastric and intestinal stages, the TP content and AC of the skin extract were found to be lower than the initial (before digestion) value. It was determined that polyphenols were more stable in gastric conditions than in the small intestine. The addition of the skin extract (0.4%) to the noodle dough increased the TP and AC of the final product compared to the noodle without the skin extract (control). It was observed that the stability of the polyphenols from the noodle sample was higher in gastric stage than intestinal one. The addition of peanut skin extract to the noodle dough increased the bioaccessibility of the polyphenols. Therefore, this study showed that peanut skin, as an important source of polyphenols, may be useful for food enrichment.

Keywords: peanut skin; polyphenol; *in-vitro* digestion; noodle

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1 Introduction

During vegetative processing, a significant amount of wastes rich in polyphenols with antioxidant activity is generated. In recent years, due to environmental factors and economic reasons, studies about valorization of these wastes have increased gradually. As a result of the processing of peanut fruit (*Arachis hypogaea* L.), a large amount of red-pink colored skin is produced as waste, and they are generally used as animal feed, fertilizer and fuel (Yu et al. 2005; Win et al. 2011). Previous studies have shown that peanut skin is a rich source of bioactive polyphenols (Larrauri et al. 2016). 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. (2014) found that the extract from potato peel waste prevented oxidation in soybean oil due to its antioxidant activity. Rashidinejad et al. (2016) 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 (Pasqualone et al. 2017). On the other hand, there is no study on the use of peanut skin extract in foods; but only, in the study conducted by Ma et al. (2014), ground peanut skin was added to peanut butter.

Although phenolic compounds have strong antioxidant activity, their bioactivity depends on their degree of bioaccessibility (Wang et al. 2017). 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 (Gullon et al. 2015), cocoa powder (Giltekin-Özgiven et

al. 2016), apple (Bouayed et al. 2012) and elderberry fruit (Pinto et al. 2017), 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 peanut skin polyphenols.

In this study, it was aimed to (1) determine the total polyphenols (TP), total flavonoids (TF) and antioxidant capacity (AC) of the extract from peanut skin, and the bioaccessibility of polyphenols (2) use peanut skin extract for enrichment in noodle production and thus to evaluate peanut skin, which is an important waste.

2 Materials and Method

2.1 Materials

The skins of peanut (*Arachis hypogaea* L.) used in the study were supplied from a peanut processing plant (Ece Tarım, Aydın, Turkey). They were stored in polyethylene bags at $4\pm 2^\circ\text{C}$ until used. Flour and eggs were obtained from the local market.

2.2 Polyphenol extraction

Polyphenols were extracted from ground powder skin and noodles (both with and without the skin extract) 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. The use of water has several advantages over commonly used organic solvents since it is an environmentally-friendly solvent with remarkable extraction capacity and no toxicity for human health (Chen et al. 2013). 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, TF, AC and bioaccessibility. Some part of the extract was also stored at -80°C for freeze-drying (at -50°C and under 0.1 mbar/0.75 mmHg vacuum) to use in noodle production. Each extraction was carried out in triplicate.

2.3 Determination of total polyphenol (TP)

TP of the extract or pure water (as a blank) was determined using the Folin-Ciocalteu method (ISO 14502-1:2005). The blue color of the reaction mixture was measured at 765 nm against blank using a spectrophotometer (Shimadzu UV-VIS 1208). A calibration curve of gallic acid (5-50 $\mu\text{g}/\text{mL}$) was prepared and the results determined from regression equation of the calibration curve ($R^2=0.99$) were expressed as mg gallic acid equivalents (GAE) per gram of dry matter (DM).

2.4 Determination of total flavonoid (TF)

The amount of TF was determined by spectrophotometric method (Rodrigues et al. 2016). Rutin (0-1500 ppm; $R^2=0.99$) was used as a standard and a standard curve was obtained with its different concentrations at 510 nm. Results were calculated based on this curve and expressed as mg rutin equivalents per gram of dry matter (mg RE/g DM).

2.5 Antioxidant capacity (AC)

AC was determined by the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) method of Türkmen Erol et al. (2009) at 517 nm. AC was calculated as percentage inhibition (AC, %) of the DPPH radical by the following equation:

$$AC(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Abscontrol: Absorbance of the solution of DPPH without sample,

Abssample: Absorbance of the solution of DPPH with sample

AC (%) of samples was converted to ascorbic acid equivalent (AAE) defined as mmol of ascorbic acid equivalents per 100 g of DM.

2.6 In-vitro digestion (Bioaccessibility)

In-vitro digestion method was applied according to Minekus et al. (2014) to evaluate the bioaccessibility of phenolic compounds of the sample extracts. It was carried out in two stages as gastric and intestinal. After each stage, the amount of TP was determined by spectrophotometer and the bioaccessibility (%) of TP was calculated as follows.

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

C digested: Concentration in digested sample after gastric/intestinal stage (mg)

C undigested: Concentration in undigested sample (mg)

2.7 Noodle production

Noodle production was carried out according to Collins and Pangloli (1997) with some modification. The freeze-dried extract was used at a rate of 0.4% in noodle production. The ratios of other ingredients were 65%, 21.6% and 13% for flour, water and egg, respectively. 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

2.8 Statistical analysis

Experimental results were expressed as means \pm 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.

3 Results and discussion

3.1 The effect of *in-vitro* digestion on TP and AC of peanut skin extract

TP and AC values of peanut skin extract at initial and after digestion are given in Table 1. TP value (71.67 mg GAE/g DM) found in this study was less than that reported by Win et al. (2011) (91.74 mg GAE/g) for peanut skin. This dissimilarity can be due to the differences in the extraction solvent, the analysis method and the standard used. When the TP value from this study was compared with the values of other plant by-products; 9.18 mg GAE/g hazelnut skin (Stevigny et al. 2007), 7.51-18.51 mg GAE/g dw (Zardo et al. 2019) for sunflower seed meal and a maximum of 1.13 mg GAE/g dw (Amado et al. 2014) for potato peel waste were reported. These results showed that the peanut skin is an important source of polyphenols. On the other hand, the result of AC (66267.46 mmol AAE/100g DM) from this study was not possible to compare to the literature values because of the fact that the same antioxidant method used in the studies was applied with different modifications and the results were expressed in different units. For example; 309 - 1375 μ mol Trolox equivalent-TE/g skin (Taş and Gökmen 2015) and 854.47- 1004.98 μ M TE/g dw (Bertolino et al. 2015) in hazelnut skin and 2077- 5214 μ mol TE/kg fresh weight (Punzi et al. 2014) in artichoke waste and 3.24 mmol TE/g extract (Vázquez et al. 2012) 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 (Fawole and Opara 2016). The reduction of TP and thus AC after gastrointestinal digestion has also been demonstrated in the previous studies with different foods. Bouayed et al. (2012) 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 (Figueroa et al. 2016).

However, unlike these results, Wang et al. (2017) 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 (Fawole and Opara 2016). 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, bioaccessibility of TP and AC values of peanut skin

	Initial	Gastric	Intestinal
TP (mg GAE/g DM)	71.67 \pm 1.32 ^a	50.00 \pm 0.59 ^b	33.41 \pm 0.73 ^a
AC (mmol AAE/100g DM)	66267.46 \pm 404.69 ^c	36276.52 \pm 463.26 ^b	25940.40 \pm 1453.22 ^a
Bioaccessibility of TP (%)	100.00 \pm 0.00 ^c	69.82 \pm 1.62 ^b	46.62 \pm 0.85 ^a

^aThe differences between means in lower case letters in the same row are significant ($p < 0.05$).

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

In order to obtain enriched noodle, peanut 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 (50.94%) 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 221.45 mmol AAE/100g DM in the enriched noodle. The results obtained from the previous studies in which noodle (Kazemi et al. 2017) and fresh noodle (Pasqualone et al. 2017) 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; Ranjha et al. 2020) and artichoke waste (0.77-1.45 mg GAE/g fresh weight; Punzi et al. 2014) 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 (Pinto et al. 2017). On the other hand, it was observed that the bioaccessibility of noodle polyphenols was higher than the peanut skin polyphenols. This could be due to binding of polyphenols to the proteins in the composition of the noodle, increasing their stability during digestion (Xiong et al. 2020). 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 was 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, bioaccessibility of TP and AC values of noodle

	Noodle	Digestion Stage		
		Initial	Gastric	Intestinal
TP (mg GAE/g DM)	Control	0.53±0.03 ^{c*}	0.29±0.01 ^b	0.12±0.01 ^a
	Enriched	0.80±0.01 ^b	0.60±0.03 ^a	0.56±0.04 ^a
Bioaccessibility of TP (%)	Control	100.00±0.00 ^c	53.75±1.81 ^b	22.27±2.64 ^a
	Enriched	100.00±0.00 ^b	74.08±3.06 ^a	69.88±5.09 ^a
AC (mmol AAE/100g DM)	Control	0	0	0
	Enriched	221.45±11.25 ^b	19.53±0.35 ^a	0

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

3.3 TF content of skin and noodles

The TF content of the peanut skin and noodle samples is shown in Table 3. More TF (123.11 mg RE/g DM) than TP was found in the peanut skin. In some previous studies, TF content was found to be 12.4–36.22 mg quercetin equivalent (QE)/g extract (Larrauri et al. 2016) in peanut shell, 47.41–166.28 mg catechin equivalent (CE)/g skin extract (Ham et al. 2015) and 12.28 mg/100 g skin extract (Lee et al. 2016) in chestnut shell. However, since the results of the researchers were expressed using different standard phenolics and in different units, a comparison could not be made with the result of this study. According to the Table 3, the addition of peanut skin extract to the noodle dough increased the TF content of the enriched noodle (328.57%) compared to the control one as in the TP content. Consistent with this result, Mir et al. (2017) observed an increase in its TF content by adding apple pulp to the composition of crackers made from rice flour.

Table 3 TF content (mg RE/g DM) of the peanut skin and noodles

Skin extract	123.11 ± 0.32 ^{c*}
Control noodle	0.35 ± 0.02 ^a
Enriched noodle	1.50 ± 0.03 ^b

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

4 Conclusions

Peanut skin was evaluated in terms of TP, bioaccessibility of polyphenols, TF and AC. Due to the high TP content of the peanut skin, the extract from the skin was freeze-dried for use in the noodle formulation. It was determined that the noodle enriched with the peanut skin extract contained higher amount of TP and TF compared to the control noodle, and therefore, the enriched noodle showed AC although it could not be detected in the control one. On the other hand, the amount of TP and AC of the skin and noodles decreased significantly 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 peanut skin can be used as a rich source of polyphenols for food enrichment.

Authors' contributions: Both authors contributed equally.

Conflict of interest disclosure:

There is no conflict of interest.

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