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Polyphenols of Artichoke Fractions and Their *In-Vitro* Digestion

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ABSTRACT

This study was aimed to determine total polyphenols (TP), total flavonoids (TF), chlorogenic acid (CA), antioxidant capacity (AC) and *in-vitro* bioaccessibility of polyphenols (as gastric and intestinal stages) of the extracts from artichoke fractions (head, bract and stalk) using different solvents (80% ethanol, 80% methanol and water). The results showed that artichoke fraction and solvent used significantly affected all parameters measured ($P<0.05$). TP and TF contents of the samples varied in the range of 1.74-5.52 mg gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM) and 1.30-7.34 mg rutin equivalents per gram of dry matter (mg RE g^{-1} DM), respectively. AC of the samples varied from 433.73 to 1243.21 mmol of ascorbic acid equivalents per 100g of dry matter (mmol AAE $100g^{-1}$ DM).TP and AC of the extracts varied depending on artichoke fraction and solvent used after *in-vitro* digestion. They were found to be lower than their initial (before digestion) values. Bioaccessibility of the polyphenols was in the range of 17.36-64.37%. CA detected in all extracts except water extracts of artichoke head (AH) and artichoke stalk (AS). These results suggest that artichoke bract (AB) and AS which are artichoke byproducts might represent a potential source of natural antioxidants as well as AH.

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Enginar Fraksiyonlarının Polifenolleri ve *In-Vitro* Sindirimi

ÖZET

Bu çalışmada, enginar fraksiyonlarından (tabla, dış yapraklar ve sap) farklı çözücüler (%80 etanol, %80 metanol ve su) kullanılarak elde edilen ekstraktların toplam polifenol (TP), toplam flavonoid (TF) ve klorojenik asit (KA) içerikleri ile antioksidan kapasitesi (AK) ve polifenollerin (mide ve bağırsak aşamasında) *in-vitro* biyoerişilebilirliğini belirlemek amaçlanmıştır. Sonuçlar, enginar fraksiyonunun ve kullanılan çözücünün ölçülen tüm parametreleri önemli ölçüde etkilediğini göstermiştir ($P<0.05$). Örneklerin TP ve TF içerikleri, sırasıyla 1.74-5.52 mg GAE (gallik asit eşdeğeri) g^{-1} KM (kuru madde) ve 1.30-7.34 mg RE (rutin eşdeğeri) g^{-1} KM aralığında bulunmuştur. AK ise 433.73-1243.21 mmol AAE (askorbik asit eşdeğeri) $100g^{-1}$ KM arasında değişmiştir. Ekstraktların *in-vitro* sindirim sonrası TP ve AK' sı enginar fraksiyonu ve kullanılan çözücüye göre değişim göstermiş ve başlangıç (sindirim öncesi) değerlerinden daha düşük bulunmuştur. Polifenollerin biyoerişilebilirliği %17.36-64.37 aralığında saptanmıştır. KA, enginar tablası (ET) ve enginar sapının (ES) su ekstraktları haricindeki tüm ekstraktlarda tespit edilmiştir. Bu sonuçlar, enginar yan ürünleri olan dış yapraklar ile sapın, ET kadar potansiyel bir doğal antioksidan kaynağı olabileceğini ortaya koymuştur.

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INTRODUCTION

Artichoke is an important vegetable consumed fresh in traditional medicine due to its pharmacological properties or canned especially in Mediterranean region countries (Dabbou et al., 2015). The artichoke plant contains health promoting compounds such as flavonoids and phenolic acids particularly caffeic acid and its derivatives (Fratianni et al., 2007; Negro et al., 2012)

Chlorogenic acid (CA) (5-caffeoylquinic acid) is one of the most abundant phenolic acids in artichoke, which has a strong antioxidant capacity (Ergezer & Serdaroğlu, 2018). During the processing of the artichoke, a large amount of vegetable waste is generated, consisting of leaves and stems surrounding it, as well as the edible part of the table. It is stated that these inedible parts, which correspond to 80-85% of the total mass, are also very rich in polyphenols (Negro et al., 2012; Dabbou et al., 2015). It is known that phenolic compounds synthesized as secondary metabolites in plants have many pharmacological properties such as antioxidant, antimutagenic, anticarcinogenic and antibacterial activity (Aires et al., 2016; Sanz-Puig et al., 2017) and have a positive effect on many chronic diseases such as diabetes, obesity and cardiovascular diseases (Ismail et al., 2021). Therefore, recently, many studies (Amado et al., 2014; Rashidinejad et al., 2016; Pasqualone et al., 2017) on 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 have been reported.

The bioavailability of a dietary compound including phenolic compound is dependent upon its digestive stability and its release from the food matrix (referred as bioaccessibility), the efficiency of its transepithelial passage (D'Antuono et al., 2015). Therefore, the beneficial effect of polyphenols is affected by their stability under gastro-intestinal conditions during digestion (Garbetta et al., 2014) due to their exposure to physiochemical changes such as temperature, pH and digestive enzymes (Pinto et al., 2017). The polyphenols bioavailability is considered to be low. Because, most of them exist in food in the form of esters, glycosides or polymers that cannot be absorbed in their native form. Only aglycones and some glucosides can be absorbed in the small intestine (Tagliazucchi et al., 2010). In this regard, the bioaccessibility of polyphenols from artichoke head were previously investigated (Garbetta et al., 2014; D'Antuono et al., 2015), but, scientific literature is still lacking on *in-vitro* gastro-intestinal digestion of polyphenols from artichoke fractions. Therefore, this study was carried out to investigate the contents of total polyphenols and CA of artichoke fractions and to assess the digestive stability of the polyphenols using *in-vitro* digestion model.

MATERIALS ve METHOD

Material

Fresh artichokes (*Cynara scolymus* L.) harvested from Bursa region (Turkey) were used in this study. Artichoke head (AH) was removed manually with the help of a knife and kept at 4±2 ºC until used, together with artichoke bract (AB) and artichoke stalk (AS) separated as waste. Chemicals used in this study were either HPLC or analytical grade and obtained from Sigma Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Polyphenol Extraction

Polyphenols from artichoke fractions homogenized in a blender (Arzum, Turkey) were extracted with aqueous ethanol (80%), aqueous methanol (80%) and distilled water. Water extracts of the samples was obtained as follows: Samples were extracted with distilled water in a controlled water bath at 1/9 (w/v) solid/liquid ratio and 50 °C for 30 min. Then, the suspension was filtered through Whatman No.1 filter paper and the extract was rapidly cooled under tap water. Methanol and ethanol extracts of the samples were obtained by the following procedure. Samples were extracted with methanol or ethanol 1/9 (w/v) solid/liquid ratio for 2 h on an orbital shaker at room temperature. The mixture was centrifuged at 10,000 rpm for 15 min and filtered through Whatman No.1. Water and alcoholic clear extracts were stored at –20 °C until analyzed.

Determination of Total Polyphenol (TP)

TP of the extracts was determined according to ISO 14502-1:2005 (2005). A calibration curve of gallic acid (5-50 µg mL^{-1}) 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).

Determination of Total Flavonoid (TF)

TF content of the extracts was determined by spectrophotometric method (Rodrigues et al., 2016). Rutin (0-1500 ppm; R^2 = 0.99) was used as a standard and standard curve was obtained with different concentrations. Results were calculated based on this curve and expressed as mg rutin equivalents per gram of DM (mg RE g^{-1} DM).

Determination of CA by HPLC

CA was determined according to Türkmen Erol et al. (2009) with some modification. The identification and

quantification of CA in the extracts 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 30 °C. UV spectra were recorded from 190-370 nm and peak area was measured at 325 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 5 min, 8% B; from 5 to 30 min, 8-10% B; from 30 to 35 min, 10-80% 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^{-1}. The injection volume was 20 µL.

Chromatographic peak in the samples was identified by comparing its retention time and UV spectrum with that of its reference standard and by co-chromatography with added standard. Quantification was performed from the peak area of the component and its corresponding calibration curve.

Antioxidant Capacity (AC)

AC was determined by the 2,2,diphenyl-2-picryl-hydrazyl (DPPH) method (Turkmen et al., 2005). AC was calculated as percentage inhibition (AC, %) of the DPPH radical by the following equation:

$$AC(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} x100 \quad (1)$$

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

Abs_{sample} : 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 100g of DM.

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 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.

$Bioaccessibility\ (\%) = (C_{digested} / C_{undigested}) \times 100$ (2)

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

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

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

TP, TF, AC and CA Content of the Extracts

The effect of artichoke fractions and solvents used on TP, TF, CA and AC values of the samples is presented in Table 1. Artichoke fraction, solvent used and their interactions significantly affected all parameters measured ($P<0.05$) (Table 2).

TP contents of the samples varied in the range of 1.74-5.52 mg GAE g^{-1} DM depending on artichoke fraction and solvent used (Table 1). When compared to the previous studies in literature, there are differences between the results. It was stated that the differences could be caused by harvest time, climatic conditions during growing of plant, the part of artichoke, extraction solvent, expression of the result using different standard phenolics and in different units, *etc.* (Ergezer & Serdaroğlu, 2018). As agreement with this result, Gaafar and Salama (2013) found 4.2-14.16 mg GAE g^{-1} DW of TP in the free and bound methanolic extracts of different parts of artichoke. However, Colantuono et al. (2018) reported higher contents of TP (8.8 - 34.7 mg GAE g^{-1} DW) from artichoke fractions (head, leaves and stem). Also, in the study of Mena-García et al. (2020), TP contents of artichoke bracts varied from 5.91 to 27.21 mg GAE g^{-1} DW, depending on the solvent used.

As seen in Table 1, in general the highest TP was observed in AH extracts, followed by AB and AS ones. Similar result was found by Mena-García et al. (2020). Regarding TP, for AH and AB, the highest TP was obtained with aqueous ethanol and water, respectively but for AS there was no significant difference between solvents ($P>0.05$). Similarly, Vella et al. (2018) reported that TP from chestnut byproducts was significantly affected by the different solvents employed, which is partially consistent with this study.

The extracts were analyzed by HPLC to determine their CA content because CA is one of the most abundant phenolic acids in artichoke. The content of CA of the samples was significantly affected by artichoke fraction and solvent used (Table 1 and 2). In general, aqueous ethanol provided the highest concentrations of CA (298.09-883.67 µg $100g^{-1}$ DM). It is also, more advantageous than methanol because it is environmentally friendly. Regarding artichoke fraction, AB contained the highest levels of CA.

TF contents of the samples varied in the range of 1.30-7.34 mg RE g^{-1} DM depending on artichoke

fraction and solvent used (Table 1). Similar result was reported by Gaafar and Salama (2013) who found 2.06-9.85 mg quercetin equivalent (QE) g^{-1} DW of TF in artichoke byproducts. However, this result is in contrast with the study of Dabbou et al. (2016) which reported that artichoke byproducts contained 7.8-64.9 mg catechin equivalent (CE) g^{-1} DW of TF. This discrepancy might be due to differences in varieties of artichoke used and expression of the results using different standard phenolics. This study showed that TF values of the extracts obtained using aqueous ethanol and methanol were lower than their TP ones but those of water extracts were higher than their TP values. Moreover, water extracted the highest amount of TF for all fractions examined. Regarding artichoke fraction, the highest levels of TF were obtained from AH extracts.

Table 1 TP, TF, CA and AC values of artichoke fractions
Çizelge 1. Enginar fraksiyonlarının TP, TF, KA ve AK değerleri

		Solvent		
		Ethanol (80%)	Methanol (80%)	Water
TP (mg GAE g^{-1} DM)	Head	$5.52 \pm 0.02^{Cb*}$	4.26 ± 0.19^{Ca}	4.63 ± 0.11^{Ba}
	Bract	3.05 ± 0.23^{Ba}	2.58 ± 0.06^{Ba}	4.68 ± 0.51^{Bb}
	Stalk	2.11 ± 0.23^{A}	1.98 ± 0.04^{A}	1.74 ± 0.14^{A}
AC (mmol AAE $100g^{-1}$ DM)	Head	1042.17 ± 1.99^{Ca}	1451.22 ± 22.80^{Cb}	1034.70 ± 12.52^{Ba}
	Bract	715.93 ± 6.81^{Ba}	751.25 ± 24.27^{Ba}	1243.21 ± 133.27^{Bb}
	Stalk	467.79 ± 6.97^{Ab}	433.73 ± 2.89^{Aa}	566.78 ± 4.03^{Ac}
TF (mg RE g^{-1} DM)	Head	5.35 ± 0.09^{Bb}	3.20 ± 0.33^{Ba}	7.34 ± 0.23^{Cc}
	Bract	1.51 ± 0.30^{Aa}	1.30 ± 0.09^{Aa}	6.25 ± 0.21^{Bb}
	Stalk	1.31 ± 0.10^{Aa}	1.61 ± 0.01^{Ab}	2.37 ± 0.04^{Ac}
CA (µg $100g^{-1}$ DM)	Head	823.40 ± 0.63^{Bc}	268.06 ± 19.05^{Ab}	-
	Bract	883.67 ± 40.25^{Bb}	862.55 ± 26.28^{Bb}	490.685 ± 36.63^{a}
	Stalk	298.09 ± 25.68^{Ac}	219.36 ± 11.23^{Ab}	-

*For each variable, means in a row (a-c across solvent type) and in a column (A-C across fraction type) with different letters are significantly different ($P < 0.05$).

Table 2 Variance analysis results on the effect of artichoke fraction, solvent and their interactions on TP, TF, CA and AC values of the extracts
Çizelge 2. Enginar fraksiyonu, solvent ve bunların interaksiyonlarının, ekstraktların TP, TF, KA ve AK değerlerine etkisine ait varyans analizi sonuçları

Sources of variation	DF[a]	TP		AC		TF		CA	
		MS[b]	F	MS	F	MS	F	MS	F
Fraction	2	12.263	127.810*	717132.080	168.529*	19.205	272.365*	510894.612	478.905*
Solvent	2	0.949	9.893*	66080.838	15.529*	18.008	255.388*	384586.121	360.505*
Fraction x solvent	4	1.183	12.329*	115429.170	27.126*	3.418	48.477*	56804.113	53.247*

[a] Degree of freedom; [b] Mean squares; * Significance at $P<0.05$

AC of the samples varying from 433.73 to 1243.21 mmol AAE $100g^{-1}$ DM is shown in Table 1. With respect to artichoke fraction, the same trend was observed as in the cases of TP and TF. As agreement with this result, Mena-García et al. (2020) found that receptacle (head) extract showed the highest antioxidant activity. While the highest AC was observed in the extracts obtained using aqueous methanol for AH, water provided the highest AC for both AB and AS among the solvents used.

The Effect of *In-vitro* Digestion on TP Content and AC of Artichoke Fractions

The TP content and AC of the extracts showed a similar trend after *in-vitro* digestion. They both greatly decreased compared to their initial values (Table 3). The reduction of TP content and thus AC after gastrointestinal digestion has also been demonstrated by D'Antuono et al. (2015) for artichoke head, Bouayed et al. (2012) for different apple cultivars and Figueroa et al. (2016) for different walnut varieties. The reduction of TP after *in-vitro* digestion could be attributed to sensitivity of polyphenols to environmental factors such as pH change, light and heat, and also their degradation by digestive enzymes (Pinto et al., 2017). In addition, during gastro-intestinal digestion, polyphenols may interact with other food constituents such as sugars, lipids, proteins and fibre, which can reduce or improve their bioaccessibility (Helal et al., 2014). Bioaccessibility of polyphenols after gastric digestion (19.54-61.62%) was almost similar to that after intestinal digestion (17.36-64.37%) depending on artichoke fraction and solvent used (Table 3). For each fraction, the highest bioaccessibility of polyphenols was observed in methanolic extract but,

the lowest one was observed in water extract. This can be due to differences in type of polyphenols in the extracts and their stabilities in relation to polarity of solvents used. Because, bioaccessibility of individual phenolic compounds was different (D'Antuono et al., 2015). Regarding artichoke fraction, in general, bioaccessibility of polyphenols in AS and AH extracts was higher than that in AB.

Table 3 Values of TP, AC and bioaccessibility of TP of artichoke fractions after *in-vitro* gastrointestinol digestion
Çizelge 3. Enginar fraksiyonlarının in-vitro gastrointestinal sindirim sonrası TP ve AK değerleri ile polifenollerinin biyoerişilebilirliği

		Gastric		
		Ethanol	Methanol	Water
TP (mg GAE g^{-1} DM)	Head	2.28 ± 0.05^{Cb}	2.50 ± 0.16^{Bb}	1.64 ± 0.07^{Ba}
	Bract	1.10 ± 0.07^{B}	1.25 ± 0.01^{A}	0.93 ± 0.20^{A}
	Stalk	0.77 ± 0.02^{Ab}	1.22 ± 0.02^{Ac}	0.52 ± 0.02^{Aa}
AC (mmol AAE $100g^{-1}$ DM)	Head	362.53 ± 13.82^{Ca}	526.04 ± 2.62^{Bb}	365.99 ± 11.17^{Ca}
	Bract	62.62 ± 7.35^{Ba}	115.45 ± 5.51^{Ab}	131.02 ± 16.36^{Bb}
	Stalk	7.98 ± 0.03^{Aa}	114.68 ± 1.77^{Ac}	58.72 ± 1.85^{Ab}
Bioaccessibility (%) of TP	Head	41.25 ± 0.75^{Aa}	58.54 ± 1.04^{Bb}	35.49 ± 2.29^{Ba}
	Bract	36.04 ± 0.33^{Ab}	48.53 ± 0.74^{Ac}	19.54 ± 2.01^{Aa}
	Stalk	36.67 ± 3.34^{Aa}	61.62 ± 2.39^{Bb}	29.78 ± 1.45^{Ba}
		Intestinal		
		Ethanol	Methanol	Water
TP (mg GAE g^{-1} DM)	Head	2.16 ± 0.00^{Cb}	2.68 ± 0.07^{Cc}	1.37 ± 0.08^{Ba}
	Bract	1.11 ± 0.01^{Bb}	1.55 ± 0.02^{Bc}	0.81 ± 0.04^{Aa}
	Stalk	0.96 ± 0.05^{Ab}	1.28 ± 0.03^{Ac}	0.68 ± 0.02^{Aa}
AC (mmol AAE $100g^{-1}$ DM)	Head	176.31 ± 22.19^{Ba}	557.75 ± 61.33^{Bb}	249.47 ± 36.90^{Ba}
	Bract	74.48 ± 6.18^{Aa}	193.77 ± 31.94^{Ab}	212.72 ± 8.01^{Bb}
	Stalk	27.48 ± 3.85^{Aa}	30.07 ± 3.26^{Aa}	107.28 ± 0.09^{Ab}
Bioaccessibility (%) of TP	Head	39.25 ± 0.08^{Ab}	62.88 ± 1.21^{Ac}	29.63 ± 2.45^{Ba}
	Bract	36.36 ± 2.65^{Ab}	60.11 ± 0.64^{Ac}	17.36 ± 1.07^{Aa}
	Stalk	45.50 ± 2.83^{Aa}	64.37 ± 2.83^{Ab}	38.83 ± 2.16^{Ca}

*For each variable, means in a row (a-c across solvent type) and in a column (A-C across fraction type) with different letters are significantly different (P<0.05).

CONCLUSIONS

In this study, TP, TF, CA and AC levels and *in-vitro* bioaccessibility of polyphenols (as gastric and intestinal stages) of the extracts from artichoke fractions were investigated. Artichoke fraction and solvent used significantly affected TP, TF, CA and AC levels of the samples. The highest TP, TF and AC values were obtained from AH extracts. However, AB contained the highest levels of CA for all solvent. For all parameters measured, efficiency of solvents used changed according to the fractions. TP content and AC at initial decreased at different rates depending on the fraction and solvent after gastrointestinal digestion. For each fraction, methanol extract had the highest bioaccessibility of TP after digestion.

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