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Determination of the in vitro bioaccessibility of phenolic compounds and antioxidant capacity of Juniper berry (*Juniperus drupacea* Labill.) pekmez

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Abstract: In this study, the total phenolic content (TPC), total flavonoid content (TFC), in vitro antioxidant capacity and individual phenolic compounds of *Juniperus drupacea* Labill berry and pekmez (molasses) were determined. Since pekmez was the only edible form of berry, the bioaccessibility of phenolic compounds and the antioxidant capacity of the pekmez were assessed following exposure to simulated gastrointestinal conditions. The phenolic compounds determined in the berry and pekmez were similar, while the pekmez was especially rich in protocatechuic acid, and additionally, the berries were rich catechin and chrysin. Generally, through oral to the intestinal stage of the simulated gastric conditions, the phenolic levels decreased. Although protocatechuic acid was the major phenolic compound at the initial stage, the highest bioaccessibility was observed for catechin and *p*-hydroxybenzoic acid. In terms of the antioxidant capacity determined by four different methods, the dialyzed fraction (IN) was 0.77-12.19% of the initial values. In this study, detailed information on the antioxidant capacity and phenolic compounds of *Juniperus drupacea* Labill pekmez and their change through the simulated gastrointestinal conditionswereevaluated for the first-time.

Key words: Bioaccessibility, in vitro digestion, *Juniperus drupacea* Labill., *Juniper* berry pekmez, phenolics

1. Introduction

Juniperus drupacea Labill. is a member of the Cupressaceae family and native to Mediterraneanregions. The berry-like fleshy cones developed by the female tree normally contain three seeds, a nut-like shell and a fleshy part that cannot be consumed as fresh fruit but used to prepare "pekmez", a type of fruit juice concentrate (Semiz et al., 2007). Stone-crushed berries are soaked in drinking water for three days to extract water-soluble solids, and afterward, the filtered extract is boiled to a concentrate. In general, 6 kg of berry fruit is used to produce 1 kg of pekmez with no added ingredients (Akinci et al., 2004). With its high energy and nutritional value, *J. drupacea* pekmez (molasses) has been used to relieve diseases such as stomachache, abdominal pain, hemorrhoids and asthma (Yesilada et al., 1993; Miceli et al., 2011).

Traditional medicine in Turkey includes the use of several *Juniperus* species (Kozan et al., 2006; Orhan et al., 2011; Seca et al., 2016). In the studies of Akkol et al. (2009) and Taviano et al. (2011), the antioxidant and antimicrobial activities of the methanol and water extracts of five *Juniperus* taxa branches growing in Turkey were compared. It was determined that both the leaf and fruit extracts showed similar in vivo antinociceptive and antiinflammatory activities, and *Juniperus oxycedrus* subsp. *oxycedrus* and *Juniperus communis* var. *saxatilis* possessed significantly higher activities than other species. Among the leaf extracts of those five species, amentoflavone and cupressuflavone were the most abundant phenolic compounds in *J. drupacea* leaves, while these leaves had the lowest content of total phenolics (Miceli et al., 2020).

The health-promoting properties of *Juniperus* species have been related to theirsecondary metabolite content and biological activities. Polyphenols constitute the primary group of natural antioxidants within the identified bioactive compounds, and there has been a rising interest in the relationshipbetween dietary antioxidant intake and reduced risk of numerous diseases such as cancer, diabetes and cardiovascular diseases (Marquardt and Watson, 2014).

There have been many in vitro studies indicating the antioxidant potential of plants containing polyphenols. However, the bioaccessibility of those compounds should also be examined since the gastrointestinal environment affects not only their stability but also their antioxidant activities. As an alternative technique to in vivo methods, in vitro digestion models for simulating gastrointestinal conditions have been extensively used since they provide

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simple, inexpensive and reproducible tools, and they have been applied for different fresh produce such as blackcurrant (Orjuela-Palacio et al., 2019), strawberry (Cervantes et al., 2019), mushroom (Ucar and Karadag, 2019), sweet cherry (Gonçalves et al., 2019) and food products such as blackcurrant juice (Uzunović and Vranić, 2008), tomato sauce (Tomas et al., 2019), strawberry juice (Cassani et al., 2018), kombucha tea beverages (Tamer et al., 2021), black carrot jams and marmalades (Kamiloglu et al., 2015), and medlar fruit leather (Suna, 2019).

In recent years, the phytochemicals of the *Juniperus* genus have been broadly studied; though only a few studieshave concentrated on *J. drupacea* Labill. berries. Miceli et al. (2011) revealed that, in berry extracts, phenolic acids constituted more than 60% of the total phenolics, and tyrosol was the major phenolic, followed by protocatechuic acid, gallic acid and chlorogenic acid, whereas, among flavonoids, amentoflavone was identified as the main compound. α-pinene, thymol methyl ether and camphor were the main constituents that exhibited clear antimicrobial activities determined in the volatile extracts of *J. drupacea* Labill. berries (El-Ghorab et al., 2008).The α-amylase and α-glucosidase inhibitory effects and antioxidant activities of the water, ethyl acetate and methanol extracts of *J. drupacea* fruits, leaves and branches were reported by Orhan et al. (2019).

Although the traditional dietary intake of *J. drupacea* Labill. berries has been associated with pekmez consumption, there have been only a few studies related to *J. drupacea* pekmez. Akinci et al. (2004) studied the nutritional composition of pekmez and berries. Izgi (2011) determined the nutritional composition and total phenolic content of twelve *J. drupacea* Labill pekmez samples produced by conventional methods. Ozdemir et al. (2004) determined the effects of processing conditions on the nutritional composition of *J. drupacea* Labill pekmez. To the best of the authors' knowledge, there have been no published data evaluating the phytochemical compositions of *J. drupacea* pekmez and the effect of in vitro gastrointestinal digestion conditions on its phenolics. Therefore, the aim of our study was to determine the phenolics and antioxidant activities of *J. drupacea* berries and pekmez and demonstrate the changes of individual phenolics, total phenolic content, total flavonoid content and antioxidant capacities when berry pekmez was subjected to in vitro gastrointestinal digestion.

2. Materials and methods

2.1. Materials

6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid (Trolox, 97%), 2,2′-azinobis (3ethylbenzthiazoline-6-sulfonic acid) (ABTS>98%) and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%), neocuproine, amylase (A1031), pepsin (P7012), pancreatin (P7545) and bile (B8631) phenolic standards and acetonitrile for high-performance liquid chromatography (HPLC) analysis) were obtained from Sigma-Aldrich LLC. (Steinheim, Germany). All other chemicals and reagents used for the analyses were of analytical grade.

2.2. Preparation of berries, pekmez (molasses) samples and extraction

J. drupacea Labill berries (Figure 1) were collected near Antalya-Akseki in Turkey by Prof. Hüseyin Fakir from the Isparta University of Applied Science, Faculty of Forestry. Pekmez (molasses) samples made out of the same *J. drupacea* Labill berries were obtained from a local producer. According to the information obtained from the producer, the berries were cracked and smashed by a hammer, put in open vessels filled with water (~1:3, w:v) and kept for three days in environmental conditions to extract soluble solids. Afterward, the extract was filtered through muslin cloths, and wood (oak tree) ash was mixed with filtered extract for clarification. The upper phase was collected by siphoning and transferred in large cauldrons hanged over an open fire, the mixture was boiled to evaporate the water, and this process continued until the desired consistency was obtained. During boiling, any foam produced was removed by using a ladle or colander.

The berries without seeds were dried at room temperature for about 2 days until the moisture content reached 23.39 ± 0.57% and powdered, and they were stored at 4 °C until the day of the analysis. Five grams of powder was homogenized (Ultra Turrax-T25, IKA, Wilmington, USA) with 50 mL of 80% aqueous methanol containing 0.1% acetic acid (v:v) for 5 min, and the mixture was placedon a magnetic stirrer for around 16 h. The supernatants were pooled after centrifugation at 2700 g and 4 °C. The residue was reextracted twice, and the methanolic extracts were combined. For extraction of pekmez, the procedure in the study by Kamiloglu et al. (2015) was followed. Ten grams of the sample was mixed with 50 mL of the same extraction solvent above, and after treatment on a magnetic stirrer for 2 h, the mixture was subjected to an ultrasonic bath (Daihan, WUC-D10H) for 15 min, centrifuged at 2700 g and 4 °C, and the supernatants were collected. This procedure was repeated again with a pellet, and the supernatants were pooled. The combined supernatants were evaporated to dryness by a rotary evaporator at 40 °C under vacuum, reconstituted to10 mL with the extraction solvent and stored at −20 °C for further analysis.

2.3. Total phenolic and total flavonoid content

The total phenolic content (TPC) of the samples was determined with the Folin-Ciocalteu (FC) reagent according to the method described by Singleton et al. (1999). Gallic acid was chosen as a reference standard. An

Figure 1. *J. drupacea* L. berries and pekmez obtained from Antalya-Akseki (Turkey).

aliquot of 0.5 mL of the extracts was added to 2.5 mL of the FC reagent (0.2N) and 2 mL of a Na_2CO_3 (2%) solution. The mixture was incubated at room temperature for 30 min in the dark, and the absorbance was measured at 760 nm using a Shimadzu 150 UV-1800 spectrophotometer (Kyoto, Japan).The results are expressed as mg gallic acid equivalent (GAE) per g of dry sample. The linear range of the standard curve was from 0.01 to 0.1 mg/mL (r^2 = 0.993).

The total flavonoid content (TFC) of the samples was determined according to the method of Zhishen et al. (1999).The extracts (1 mL) were mixed with 4 mL of distilled water, 0.3 mL of $NaNO_2$ (5%), 0.3 mL of an $AlCl_3$ (10%) solution, and they were left for 6 min. Then, 2 mL ofNaOH (1M) was added, and the volume was completed to 10 mL with distilled water. The absorbance was measured at 510 nm using a spectrophotometer. The results are presented as mg catechin equivalent (CAE) per g of dry sample. The linear range of the standard curve was from 0.01 to 0.35 mg/mL (r^2=0.996).

2.4. Antioxidant capacity assays

The scavenging activity of the samples against theDPPH radicalwas evaluated according to the method of Brand-Williams et al. (1995). Volumes of 0.1 mL of the extracts were mixed with 4.9 mL of a DPPH solution (0.1 mM in methanol). The mixture was incubated at room temperature for 20 min in the dark. The absorbance was measured at 517 nm by a spectrophotometer, and the results are expressed as mg Trolox equivalent (TE) per g ofdry sample. The curve for the Trolox was linear in the concentration range of 0.05–0.8 mg/mL (r^2 = 0.994).

The method described by Apak et al. (2004) was followed for the determination of the cupric-reducing antioxidant capacity (CUPRAC) of the samples. 1-mL portions of $CuCl_2$ (0.01 M), neocuproine (7.5 mM) and ammonium acetate buffer (1 M, pH 7.0) were mixed. After addition of 0.1 mL of the extractand 1 mL of distilled water, the mixture was incubated at room temperature for an hour in the dark. The absorbance was measured at 450 nm using a spectrophotometer, and the results are expressed as mg TE per g ofdry sample. The standard curve ranged from 0.025 to 0.8 mg/mL (r^2 = 0.996).

The FRAP (ferric reducing antioxidant power) assay was performed according to the method described by Benzie and Strain (1996). One hundred microliters of the extract was mixed with 900 µL of water and 2 mL of the FRAP reagent and incubated at room temperature for 30 min in the dark. The absorbancewas measuredat 593 nm using a spectrophotometer. The results are expressed as mg Fe^{2+} equivalent (Fe^{2+}E) per g of dry sample. The standard curve ranged from 0.008 to 0.5 mg/mL (r^2 = 0.999).

The ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) radical stock solution (7 mM) was mixed with potassium persulfate (2.45 mM), allowing the mixture to wait in dark at room temperature for 12–16 h, and the mixture was diluted with distilled water to an absorbance of 0.700 before use. 0.1 mL of theextractwas mixed with 2 mL of the diluted ABTS solution, and the absorbance was measured at 734 nm 6 min after initial mixing (Re et al., 1999). The results are expressed as mg Trolox equivalent (TE) per g of dry sample. The standard curve ranged from 0.003 to 0.175 mg/mL (r^2 = 0.989).

2.5. HPLC analysis of phenolic compounds

The phenolic profiles of the samples were evaluated using an HPLC system (LC-20AD pump, SIL-20A HT autosampler, CTO-10ASVP column oven, DGU-20A5R degasser and CMB-20A communications bus module) coupled to a diode array detector – SPDM20A DAD (Shimadzu Corp., Kyoto, Japan). The external standards of gallic acid, protocatechuic acid, p-hydroxybenzoic acid, catechin, caffeic acid, syringic acid,ellagic acid, o-coumaric acid, m-coumaric acid, rutin, ferulic acid, myricetin, quercetin, kaempferol and chrysin were used for preparation of the standard calibration curves. Identification and quantitative analyses were carried out based on the retention times and external standard curves. The extracts were filtered through a 0.45-µm membrane filter. Separations were conducted at 40 °C on a reversed-phase Intersil ODS C-18 column (250 mm × 4.6 mm length, 5 µm particle size, GL Sciences, Tokyo, Japan). The mobile phase included solvent A (distilled water with 0.1% (v/v) acetic acid) and solvent B (acetonitrile with 0.1% (v/v) acetic acid). A gradient elution was applied as follows: 10% B (0 to 2 min), 10% to 30% B (2 to 27 min), 30% to 90% B (27 to 50 min) and 90% to 100% (51 to 60 min) and at 63 min returns to the initial conditions. The flow rate was 1 mL/min.The chromatograms were assessed at three different wavelengths (278, 320 and 360 nm). The HPLC-DAD results are expressed as µg of individual phenolic per g ofdry sample (berry or pekmez).

2.6. Simulated in vitro gastrointestinal digestion assay

Simulated in vitro gastrointestinal digestion was performed according to the method described by Brodkorb et al. (2019), Dantas et al. (2019) and Minekus et al. (2014). To simulate the oral phase, 5 g of pekmez was mixed with 5 mL of oral phase at a ratio of 1:1 (w:w). The oral phase consisted of a simulated salivary fluid (SSF, 3.5 mL), α-amylase solution (0.5 mL, 75 U/mL), $CaCl_2$ (25 µL, 0.75 mM) and a required amount of water. The mixture was then adjusted to pH 7, and tubes were incubated at 37 °C for 2 min at 100 rpm. The oral bolus was mixed with simulated gastric fluid (SGF), $CaCl_2$ (0.075 mM), pepsin solution (1.6 mL, 2000 U/mL) and the necessary amount of water. The HCl (1M) solution was used to adjust the pH to 3, and the final ratio of the oral bolus to the SGF was 1:1. The beaker was incubated at 100 rpm for 2 h at 37 °C. Afterward, the gastric chyme was mixed with simulated intestinal fluid (SIF), $CaCl_2$ (0.3 mM), pancreatin solution (100 U/mL), fresh bile (10 mM) and the necessary amount of water. A NaOH (1M) solution was used to adjust the pH to 7, and the final ratio of the gastric chyme to the SIF was 1:1.The segments of dialysis bags (MWCO 12,000 Da) placed in the SIF medium were filled witha sodium bicarbonate (0.1 M) solutionand incubated at 100 rpm for 2 h at 37 °C. The content of the dialysis bags was the compoundsthat entered the serum (IN), and the content outside the bags was showingthe material that remained in the GI tract (OUT). The supernatants of the samples taken for the oral, gastric and intestinal phases were collected after centrifugation at 4200 rpm at 4 °C. A blank test tube without pekmez but with all simulated digestion fluids was subjected to analysis and used for the spectrophotometric assays. All lyophilized supernatants were kept at −20 °C until further analysis. Bioaccessibility (BI%) was determined as described by Dantas et al. (2019).

$$Bioaccessibility\ (BI)\ \% = \frac{dialyzed\ fraction\ (IN)}{non\ -\ digested\ sample} \times 100$$

2.7. Statistical analysis

All experiments were performed in triplicates, and the resultsare expressed as mean ± standard deviation.The differences of the properties between *J. drupacea* berry and pekmez were evaluated by *t*-test, the differences among the concentrations of individual phenolics, TPC, TFC and antioxidant activity values obtained at different steps of the in vitro digestion assay were analyzed by one-way ANOVA, and Tukey's posthoc test was applied for the comparisons of the means between the groups (SPSS version 20, IBM Corporation, Armonk, NY, USA). The differences were considered significant if $p < 0.05$.

3. Results and discussion

3.1. Total phenolic content, Total flavonoid content and individual phenolics

The total phenolic and total flavonoid content values of the *J. drupacea* Labill berries and pekmez are given in Table 1. The total phenolic content (TPC) of the berries was found as 2.50 ± 0.23 mg GAE/g in dried berries and lower than the content determined by Miceli et al. (2011) (10.24 ± 0.21 mg GAE/g). The difference could have resulted from the ripeness of the berries, the location where they were picked up, storage and extraction conditions applied. Taviano et al. (2011)determined the total phenolic content of *J. drupacea* Labill branch extract as 11.79 ±0.28 mg GAE/g. On the other hand, in the pekmez sample made out of *J. drupacea* Labill berries, the TPC increased to 4.06 ± 0.10 mg GAE/g. In comparison to another fruit pekmez studied by Kamiloglu and Capanoglu (2014), this value was higher than those of grape, white mulberry and carob pekmez (0.9–2.86 mg GAE/g) and similar to black mulberry pekmez (4.66 ±0.19 mg GAE/g). Izgi (2011) determined the range of TPC between 0.95 and 2.1 mg GAE/g for twelve *J. drupacea* Labill berry pekmez samples collected from the Hatay city of Turkey. The total flavonoid content (TFC) of the *J. drupacea* Labill berries and pekmez was determined as 0.85 ± 0.02 and 2.05 ± 0.22 mg CE/g, respectively. The TFC of *J. drupacea* Labill pekmez was found higher than those determined for grape, carob, white and blackberry pekmez (0.14-1.05 mg CE/g) (Kamiloglu

Table 1. Total phenolic content, total flavonoid content and antioxidant activity values of *J. drupacea* Labill. berries and pekmez.

	Unit	Berry	Pekmez
Total dry matter	g/100g	76.61 ± 0.57[a]	79.82 ± 0.47[b]
TPC	mg GAE/g	2.50 ± 0.23[a]	4.06 ± 0.10[b]
TFC	mg CE/g	0.85 ± 0.02[a]	2.05 ± 0.22[b]
DPPH	mg TE/g	3.89 ± 0.03[a]	3.84 ± 0.22[a]
ABTS		51.31 ± 3.57[a]	65.70 ± 1.54[b]
CUPRAC		8.20 ± 1.18[a]	26.41 ± 0.26[b]
FRAP	mg Fe^{2+}E/g	5.85 ± 0.09[a]	12.98 ± 1.53[b]
Phenolics			
Gallic acid	µg/g	40.53 ± 9.95[a]	44.68 ± 2.36[b]
Protocatechuic acid		77.01 ± 19.65[a]	251.65 ± 25.94[b]
Catechin		41.38 ± 11.04[b]	28.77 ± 9.59[a]
p-hydroxybenzoic acid		23.06 ± 7.86[b]	8.06 ± 2.89[a]
Syringic acid		13.55 ± 1.15[b]	1.61 ± 0.46[a]
m-coumaric acid		5.25 ± 1.54[b]	2.51 ± 1.01[a]
Chrysin		74.44 ± 11.48[b]	21.33 ± 5.32[a]
Rutin		11.02 ± 3.19[a]	16.70 ± 3.66[b]
Total		287.28 ± 17.28[a]	372.11 ± 45.72[b]

The results are expressed as mean ± S.D. of triplicate measurements. Means with different letters in the same row are significantly different ($p < 0.05$). GAE: gallic acid equivalent, CE: catechin equivalent, TE: Trolox equivalent, Fe^{2+}E: Fe^{2+} equivalent. TPC: total phenolic contents, TFC: total flavonoid contents, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, CUPRAC: copper reducing antioxidant capacity, ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline6-sulphonic acid), FRAP: ferric reducing antioxidant power. The results are expressed for per g of berry and pekmez (dry weight).

and Capanoglu, 2014). Gallic acid, protocatechuic acid, catechin, p-hydroxybenzoic acid, syringic acid, m-coumaric acid, chrysin and rutin were identified in both the *J. drupacea* Labill. berries and pekmez samples (Figure 2). In the study by Miceli et al. (2011), the presence of gallic acid, protocatechuic acid, chlorogenic acid, catechin and rutin in *J. drupacea* L. berries was also reported. They also reported the presence of tyrosol and amentoflavone as the major phenolics in addition to the aforementioned phenolic acids. Although the chromatogram of pekmez and berry extracts had unidentified peaks in common, their exact identification could not be established since we did not have the related external standards.

While the pekmez sample was especially rich in protocatechuic acid, the berries were rich in catechin and chrysin (Table 1). The impacts of different food processing operations on the phytochemicals of different fruit, vegetable and grain products have been reported (Nayak et al., 2015). Thermal processing applied during pekmez production could cause disruption of cell walls, provide better extractability, breakthe chemical bonds of higher molecular weight polyphenols, and therefore, result in the presence of a higher amount of free protocatechuic acid in pekmez. Hydroxybenzoic acid derivatives (gallic acid, protocatechuic acid, vanillic acid, syringe acid, p-hydroxybenzoic acid, etc.) form as sugar derivatives and alsogenerally present as the bound form in plant cells and hydrolysable tannins (Nayak et al., 2015). In the study by Mikulic- Petkovsek et al. (2020), different products (jam, liqueur, juice and tea) obtained from juneberry (*Amelanchier lamarckii*) fruits and their phenolic contents were compared. Processing of the fruits significantly affected the phenolic compounds, and compared to the control fruits, the jam had 14% higher phenolic content, and a generally higher amount of individual phenolic compounds was reported in jam and liqueur products. Zoubiri et al. (2019) observed that traditional drying and homemade jam processing increased the contents of gallic acid hexose and protocatechuic acid-hexoside compared to the fresh grapes. Similarly, Silva et al. (2019) reported

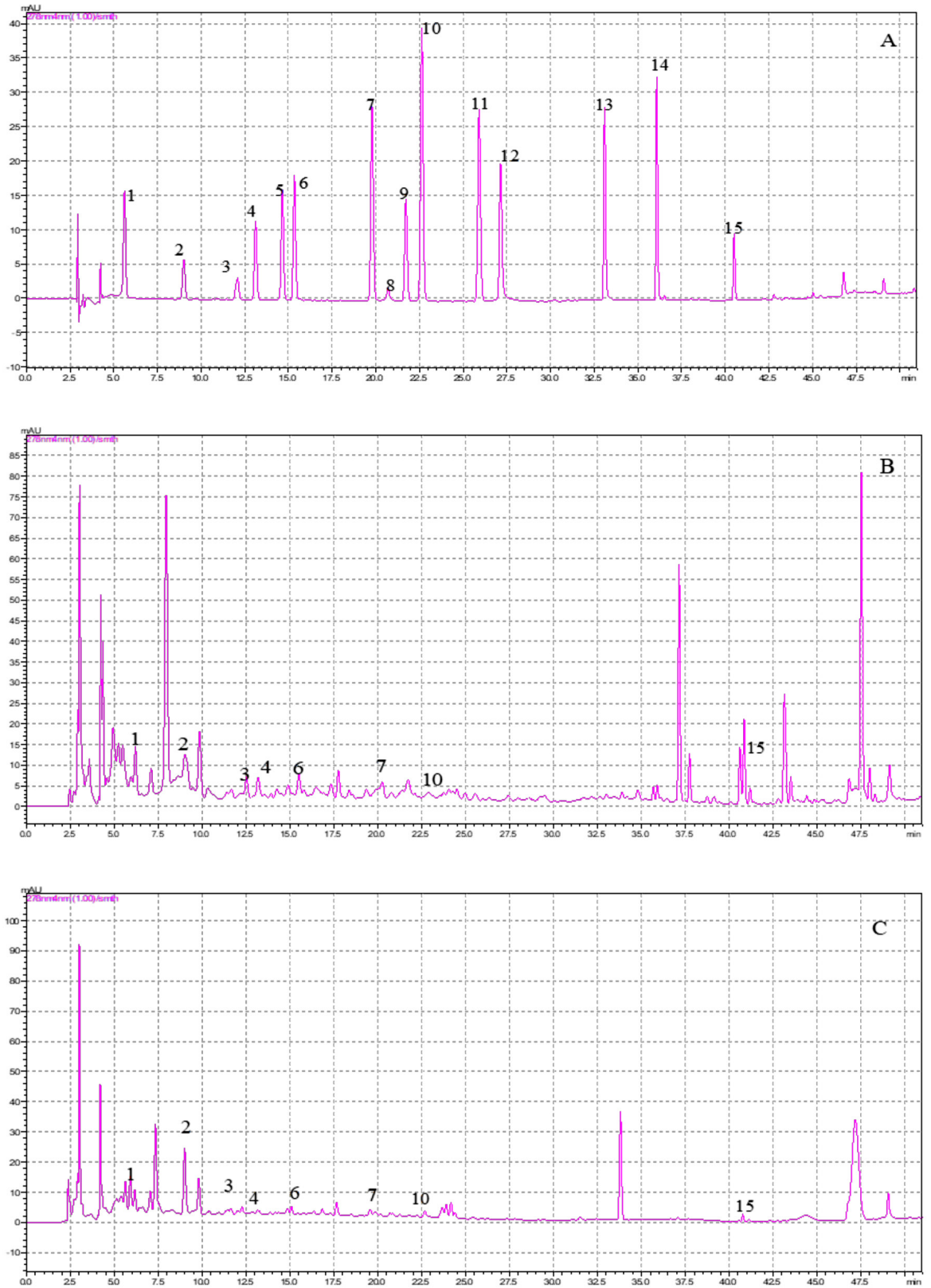

Figure 2. HPLC chromatogram for the standard mixture (A) and methanolic extract of *J. drupacea* L. berries (B) and pekmez (C) 1: gallic acid; 2: protocatechuic acid; 3: catechin acid; 4: p-hydroxybenzoic acid; 5: caffeic acid; 6: syringic acid; 7: rutin; 8: ellagic acid; 9: ferulic acid; 10: m-coumaric acid; 11: o-coumaric acid; 12: myricetin; 13: quercetin; 14: kaempferol; 15: chrysin.

that applying heat for production of grape juice resulted in higher amounts of bioactive compounds either due to the release of bound phenolicsor inactivation of enzymes responsible for phenolic degradation.

The change in the TPC and TFC of *J. drupacea* Labill pekmez is presented in Table 2. The difference of the TPC and TFC between the methanolic pekmez extracts and oral pekmez bolus could be ascribed to the presence of the alpha-amylase enzyme in the simulated salivary fluid. It is very well known that the majority of polyphenols including flavonoids in plants are found as glycosidic conjugates with sugar residues. Glycoside hydrolases including amylases can catalyze hydrolysis of glycosidic groups that are formed between a carbohydrate and a noncarbohydrate (Ara et al., 2013), and therefore,an enhanced release of polyphenols from the matrix could be observed. Similar to our results, in the study by Lucas-González et al. (2018), after the oral digestion phase,the phenolic acid concentration in persimmon fruit flours was increased up to 176.7%. Accordingly, compared to the methanolic pekmez extracts, the content of individual phenolics was also higher in the oral pekmez bolus.

In many studies, the oral digestion step was omitted (Bouayed et al., 2012; Kamiloglu and Capanoglu, 2014; Mosele et al., 2016; Seraglio et al., 2017), and compared to the initial amount in the undigested samples, mostly an elevated amount of total phenolic and flavonoid contents after the gastric digestion step was reported. This could be related to liberation of phenolics bound to fiber or proteins due to exposure of the food matrix to enzymes (e.g., pepsin) and acidic conditions (Chen et al., 2014; Lucas-González et al., 2018; Ucar and Karadag, 2019). In our sample, after gastric digestion, the amounts of TPC and TFC werehigher than those of the methanolic extract, though they were reduced compared to those of the oral phase. The reduction of the content in major phenolics (protocatechuic acid, catechin and gallic acid) ranged from 22% to 40%. Similar to our results, compared to the oral bolus, some level of reduction of phenolics after the gastric step was also reported by Chait et al. (2020) and Czubinski et al. (2019). The reason for not observing an additional increase of phenolics after the gastric step in our samples could be related to the very low level of the protein and fiber contents of pekmez, so that the action of the gastric

Table 2. Change in the total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacities, individual phenolics and bioaccessibility index of pekmezphenolics during in vitro gastrointestinal digestion

		Oral	Gastric	Intestinal		BI %
				IN	OUT	
TPC	mg GAE/g	7.70 ± 0.28^{c}	6.36 ± 0.33^{b}	0.23 ± 0.01^{a}	6.46 ± 0.38^{b}	5.59 ± 0.30
TFC	mg CE/g	6.24 ± 0.61^{b}	4.23 ± 0.51^{a}	n.d.	3.93 ± 0.74^{a}	-
DPPH	mg TE/g	24.63 ± 1.52^{d}	11.89 ± 0.67^{b}	0.47 ± 0.07^{a}	18.42 ± 1.22^{c}	12.19 ± 1.83
ABTS		22.49 ± 0.67^{d}	18.05 ± 1.25^{c}	0.51 ± 0.03^{a}	14.95 ± 0.47^{b}	0.77 ± 0.05
CUPRAC		56.08 ± 0.89^{d}	35.58 ± 2.82^{b}	0.60 ± 0.16^{a}	42.77 ± 0.71^{c}	2.29 ± 0.60
FRAP	mg Fe^{2+}E/g	35.77 ± 2.31^{d}	29.54 ± 2.96^{c}	0.73 ± 0.15^{a}	16.23 ± 1.81^{b}	5.60 ± 1.19
Phenolics						
Gallic acid	µg/g	160.98 ± 22.81^{b}	105.87 ± 39.85^{b}	9.02 ± 1.50^{a}	11.21 ± 3.49^{a}	20.18 ± 3.36
Protocatechuic acid		486.53 ± 64.62^{b}	375.94 ± 44.90^{b}	21.87 ± 8.92^{a}	64.80 ± 17.60^{a}	8.69 ± 3.55
Catechin		91.40 ± 10.58^{c}	55.25 ± 13.59^{b}	27.73 ± 1.74^{a}	8.81 ± 2.78^{a}	96.38 ± 6.05
p-hydroxybenzoic acid		37.22 ± 6.84^{c}	18.15 ± 1.54^{b}	7.55 ± 1.91^{ab}	1.93 ± 0.76^{a}	93.73 ± 23.77
Syringic acid		2.56 ± 1.22^{a}	1.85 ± 0.39^{a}	0.76 ± 0.32^{a}	1.01 ± 0.27^{a}	47.40 ± 20.17
m-coumaric acid		5.44 ± 1.14^{a}	5.13 ± 0.67^{a}	n.d	n.d	-
Chrysin		17.24 ± 0.84^{c}	13.65 ± 1.79^{b}	7.64 ± 0.39^{a}	6.78 ± 1.09^{a}	35.81 ± 1.84
Rutin		26.30 ± 5.38^{b}	22.63 ± 3.37^{b}	8.88 ± 1.02^{a}	7.21 ± 1.96^{a}	53.18 ± 6.16
Total		*827.67 ± 74.64^{c}*	*598.46 ± 54.20^{b}*	*83.46 ± 12.98^{a}*	*101.76 ± 18.83^{a}*	*22.24 ± 3.46*

The results are expressed as mean ± S.D. of triplicate measurements. Means with different letters (a–d) in the same row are significantly different ($p < 0.05$). GAE: gallic acid equivalent, CE: catechin equivalent, TE: Trolox equivalent, Fe^{2+}E: Fe^{2+} equivalent, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, CUPRAC: copper reducing antioxidant capacity, ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline6-sulphonic acid), FRAP: ferric reducing antioxidant power, BI%, bioaccessibility index. The results are expressed for per g of pekmez (dry weight).

fluid would be negligible in terms of affecting the presence of phenolics bounded to protein or fiber. The protein content of *J. drupacea* Labill pekmez was reported very low as 0.72 ± 0.04% (Akinci et al., 2004), and the traditional pekmez production process includes the clarification step that would eliminate fibers (Karababa and Işikli, 2005) and possibly any phenolic compounds bound to their structure.

At the intestinal stage, the TPC and TFC did not alter significantlyin comparison to the previous step. In the study by Kamiloglu and Capanoglu (2014), after the intestinal stage, the TPC and TFC of all pekmez samples did not decreased, and they even increased in black and white mulberry pekmez samples. O'Sullivan et al. (2013) compared the antioxidant capacity of commercial honey before and after in-vitro digestion and revealed that,compared to the initial amount in the honey, there was no significant change in TPC in digestates. Moreover, after duodenal digestion (Seraglio et al., 2017), honey samples showed a significant decrease in TPC values in comparison to gastric and initial values, and this difference could be related to the food matrix and minor differences in the digestion protocols applied. It was reported that the high sugar content in samples such as jam may affect dialysis rates, and therefore, diffusion of phenolics(Gil-Izquierdo et al. 2002). Additionally, the Folin-Ciocalteu assay used for determination of TPC is highly subjected to interferencesthat could be formed due to the action of enzymesand pH inthe intestinal conditions;therefore, this assay alone may not be adequate to observe the changes of phenolics during in vitro digestion (Tagliazucchi et al., 2010).

While the individual phenolics in our samples were evaluated,a significant reduction was observed after the intestinal stage (the sum of the IN and OUT fractions) (Table 2). In previous studies, the instability of phenolics under alkaline conditions was already reported, this might be associated with oxidation and polymerization reactions thatoccurred, and polyphenol compounds could also be converted into different structural forms with various chemical properties that were undetectable by the detection method that was applied (Chen et al., 2014; Lucas-González et al., 2018). The bioaccessibility indices of the individual phenolics varied between 8.69% and 96.38% in our samples. These values reflected the ratios of the phenolics that could pass through the simulated intestinal barrier in the intestinal fraction. Although the highest amount of phenolics detected in the pekmez and digesta was in protocatechuic acid, it had the lowest bioaccessibility index. In the study by Dutra etal. (2017), protocatechuic acid was one of the major phenolic acids in fruit pulps, but it presented very low bioaccessibility (0.8%–1.88%).

The highest bioaccessibility was determined for catechin (96.38%) and *p*-hydroxybenzoic acid (93.73%) in our samples. In previous studies employing a semipermeable cellulose membrane, the bioaccessibility of catechin varied between 19.53% and 270.71% depending on the food matrix and initial phenolic composition (Dutra et al., 2017; Dantas et al., 2019). It was suggested that the high bioaccessibility of catechins could have resulted from partial hydrolysis of proanthocyanidins which are made of catechin and epicatechin when exposed to intestinal pH values (De Morais et al., 2020). The bioaccessibility of rutin in our samples was 53.18%, and it was comparable to the amount (7.06%–41.34%) reported by Dantas et al. (2019). The significant amount of phenolics determined inthe OUT fraction may be related to metabolization by colonic bacteria (Tomas-Barberan et al., 2018).

3.2. Antioxidant capacity

It is considered that at least two assays should be applied in assessing the antioxidant capacities of samples (Kamiloglu et al., 2015), because each method has a different reaction mechanism to exert an antioxidant effect (Karadag et al., 2009). Therefore, in our study, four different assays were employed, and the antioxidant capacities of the *J. drupacea* Labill. berries and pekmez are given in Table 1. The free radical scavenging activity was evaluated by the $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals. The DPPH radical scavenging activity value of the berries in our study wasdetermined as 3.89 ± 0.04 mg TE/g berry. Miceli et al. (2011) reported the EC50 values of DPPH test for berry extract and standard BHT as 0.38 ± 0.02 mg/mL and 0.067 ± 0.00 mg/mL, respectively. In the study by El-Ghorab et al. (2008), the TBHQ (100 µg/mL) standard and *J. drupacea* Labill. berry ethanol extract (200 µg/mL) exhibited similar DPPH radical scavenging activity. In comparison to the DPPH radical scavenging activity of other fruit pekmez, the *J. drupacea* Labill. berry pekmez had higher activity (3.84 ± 0.22 mg TE/g) than grape, carob and white mulberry pekmez (0.52–2.95 mg TE/g) and lower activity than black mulberry pekmez (4.11 ± 0.14 mg TE/g). The ABTS radical scavenging capacity of the berry was determined as 51.31 ±3.57 mg TE/g. Orhan et al. (2019) reported that the ABTS radical inhibition capacity was 32.11 ± 2.37% at 3 mg extract/mL, the extract yield was reported as 26.89%, and therefore, this value would correspond to 11.15 mg of berry. The ABTS value of the *J.drupacea* Labill berry pekmez (65.70 ± 1.54) was found higher than those of other fruit pekmez reported in the literature (Kamiloglu and Capanoglu, 2014). The CUPRAC and FRAP assays are based on reduction of Cu (II) and Fe (III) ions by the action of antioxidants and performed at pH 7 and 3, respectively. The pekmez sample showed higher reduction power in comparison to the berries in both methods (Table 1). Although the method was different, moderate Fe^{2+} chelating abilities of berry extract were reported by Miceli et al. (2011) and Orhan et al. (2019).

The change in theantioxidant capacity values of the pekmez when exposed to in vitro digestion is presented

in Table 2. In comparison to the methanolic extract, the antioxidant capacity values of the oral bolus determined by all assays except ABTS showed increased values, correspondingly to the increased amount of phenolics in the oral bolus. The presence of the amylase enzyme could provide conversion of the aglycone forms of phenolics. In many studies, it has been proven that polyphenols glycosides (with single or multiple sugar moieties) have lower antioxidant activities than respective aglycones (Heim et al., 2002; Gawlik-Dziki, 2004).

At thegastric digestion step, all antioxidant capacity values decreased significantly ($p < 0.05$), and the highest decrease was observed in theDPPH value. Decreases in DPPH values in fruit pekmez and honey following gastric digestion were also reported by Kamiloglu and Capanoglu (2014) and O'Sullivan et al. (2013). After intestinal digestion, the DPPH and CUPRAC values significantly increased, and the ABTS and FRAP values decreased in comparison to the gastric step. A similar trend was observed in theDPPH and FRAP values of digested honey samples in the study by Seraglio et al. (2017). These behaviors could have been related to the changes as a result of the interaction of the food matrix with digestive fluid components, the digestion conditions (especially pH and enzymes) theinteraction of polyphenols with other components of the matrix such as minerals, and formation of new compounds and complexes that could alter the chemical structure, solubility and possess different antioxidant properties.

4. Conclusion

This study investigated the phenolic contents and antioxidant capacities of Juniper berry (*Juniperus drupacea* Labill) and pekmez and the effects of in vitro digestion on the antioxidant capacity and phenolics. The oral pekmez bolus showed higher amounts of phenolics and antioxidant capacities compared to the methanolic extract, which would suggest hydrolysis of glycosylated phenolic compounds especially in sugar-rich products and liberation of free phenolics from the food matrix. While the pekmez and berries were especially rich in protocatechuic acid, the berries were additionally rich incatechin and chrysin. Generally, through the oral to the intestinal stage of the simulated gastric conditions, the phenolic levels decreased. Although protocatechuic acid was the major phenolic at the initial stage, the highest bioaccessibility was observed for catechin and *p*-hydroxybenzoic acid. Therefore, apart from the initial amount of phenolics determined in the food sample, their fate through digestion was important to consider their possible health-promoting effects upon dietary consumption.In this study, only one pekmez formulation produced by the conventional method was assessed to understand the bioaccessibility of phenolics from the pekmez matrix. However, for future studies, the effects of industrial processing conditions may be investigated for multiple samples produced with different methods. Although the highest amount of phenolics detected in the pekmez and berry was protocatechuic acid, it had the lowest bioaccessibility index. The phenolic composition of *Juniperus drupacea* Labill. pekmez and the in vitro bioaccessibility of phenolics should be included to adapt novel pekmez formulations into industrial production.

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Conflict of interest

The authors have declared no conflicts of interest for this manuscript.

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