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Characterization of Pekmez Samples Produced with Different Fruits According to Sugar, Organic Acid, Antioxidant Activities, and Moisture Contents

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Highlights:
- Glucose and fructose content in pekmez
- Organic acids
- Antioxidant activities

Keywords:
- Glucose
- Fructose
- Organic acid
- DPPH
- Total phenolic contents

ABSTRACT:

Chemical properties of grape, cranberry, blueberries, pomegranate, black mulberry, juniper, and carob pekmez samples were investigated in this study. Capillary electrophoresis was used to assess the organic acids and sugar levels of pekmez samples. The two main sugars were found to be as glucose and fructose. Juniper pekmez has the lowest sugar with 22.6 g /100 g glucose and 20.1 g /100 g fructose. Black mulberry (35.6 g/100 g) has the highest glucose whereas grape (45.8 g /100 g) includes the highest fructose. Each pekmez samples included 8 organic acids which are oxalic, tartaric, malic, citric, succinic, acetic, lactic, and gluconic acid. 1,1-diphenyl-2-picryl-hydrazil (DPPH) technique was applied for testing the antioxidant activities. Carob pekmez scavenged DPPH the most with 96.28% inhibition value. The other pekmez samples have inhibited DPPH with the values ranging between 58.04% (cranberry) to 90.16 (juniper). The samples' total phenolic levels ranged from 265 mg GAE/g (blueberry) to 1070 mg GAE/g (grape). Moisture contents were measured using a moisture analyzer. Cranberry pekmez (19.06%) and grape pekmez (12.24%) have significantly the highest moisture compared to other samples changing between 2.41% and 5.10%.

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INTRODUCTION

Anatolia has a rich cuisine due to its diverse culture, a wide variety of dishes, and unique ingredients. One of these special and widely used traditional Anatolian food is pekmez. Pekmez is a concentrated version of various fruit juices that contains up to 65-80% soluble dry matter (Karababa et al., 2005; Turkben et al., 2016). It is made by traditional or technology methods of crushing and boiling a variety of fruits without the addition of sugar or other culinary additives. It is eaten as a dessert either plain or with tahini. It is also utilized in conventional foods like halva.

The main fruit used in pekmez manufacturing is grape. The Turkish Statistical Institute estimates that 4 200 000 tons of grapes are produced in Turkey each year (Anonymous, 2021) and 40% of this amount is used for the production of grape pekmez. Besides grape, several fresh or dried fruits such as fig, mulberry, apple, apricot, cranberry, blueberries, pomegranate, black mulberry, juniper, and carob which contain a high level of sugar are also used in making pekmez.

Pekmez is an energy source rich in carbohydrates, mixes into the blood shortly after consumption without the need for digestion due to the monosaccharides (Şimşek and Artik, 2002; Sengül et al., 2005; Akbulut and Özcan, 2008; Aliyazicioglu et al., 2009; Turkben et al., 2016; Salık et al., 2021). Pekmez is nutritionally crucial, especially for infants, kids, athletes, and circumstances requiring a quick source of energy. Pekmez also provides bone development due to its iron and calcium content and is good for anemia (Demirözü et al., 2002; Şimşek and Artik, 2002; Sengül et al., 2005; Akbulut and Özcan, 2008; Akbulut and Özcan, 2009; Turkben et al., 2016). When consumed in cold weather, it provides the heat balance of the body, strengthens the immune system, and protects the body against diseases due to its antioxidant effect (Alasalvar et al., 2005; Karababa et al., 2005; Aliyazicioglu et al., 2009; Turkben et al., 2016, Salık et al., 2021).

Studies on the chemical and physical characteristics of pekmez often only discuss grape and mulberry pekmez (Demirözü et al., 2002; Kaya and Belibağlı, 2002; Sengül et al., 2005; Akbulut and Özcan, 2009; Aydinlik and Battaloglu, 2016; Turkben et al., 2016; Salık et al., 2021). The results in some of these reports were evaluated according to the geographic and botanical origins of the fruits which pekmez was produced from (Kaya and Belibağlı, 2002; Demirözü et al., 2002; Turkben et al., 2016; Salık et al., 2021). There are a few studies on the bioactivities and chemical ingredients of this important Anatolian food product produced with different fruits such as fig (Şimşek and Artik, 2002), carob (Şimşek and Artik, 2002, Yoğurtçu and Kamışlı, 2006; Sengül et al., 2007; Aliyazicioglu et al., 2009), sorghum (Akbulut and Özcan, 2008), date and apricot (Aliyazicioglu et al., 2009), cherry laurel (Alasalvar et al., 2005), rosehip (Yoğurtçu and Kamışlı, 2006) beside grape and mulberry. It is significant to remember that the nutritional content of pekmez could vary according to the fruit from which it is produced.

The purpose of this study was to analyze seven pekmez samples made from grape, cranberry, blueberry, pomegranate, black mulberry, juniper, and carob to evaluate their sugar, organic acid, antioxidant activities, and moisture contents. This is the first scientific report on cranberry, blueberry, pomegranate, black mulberry, and juniper pekmez. Moreover, for the first time, organic acids in pekmez and their moisture contents were reported in this study.

MATERIALS AND METHODS

Materials

Sigma-Aldrich provided D(+) fructose and D(+) glucose (Steinheim, Germany). Supelco provided the acetic, citric, L-(+)-lactic, D-(+), malic, oxalic, D-(-), tartaric, and succinic acids (Bellefonte, Pennsylvania, USA). The following chemicals were from Merck (Darmstadt, Germany): sodium carbonate anhydrous, 2,6-pyridinedicarboxylic acid, sodium hydroxide, 1,1-diphenyl-2- picrylhydrazil, D-gluconic sodium salt, N-cetyl-N,N,N-trimethylammonium bromide (CTAB). Glycyl-glycine and the phenol reagent of Folin-Ciocalteu were obtained from Fluka (Buchs, Switzerland).

Seven different kinds of pekmez samples namely grape, black mulberry, cranberry, pomegranate, carob, blueberry, and juniper were purchased from supermarkets in İstanbul, Turkey.

Experimental procedure

Agilent 1600 capillary electrophoresis device combined with an ultraviolet diode array detector (UV-DAD) (Waldbronn, Germany) was used. Data were processed via the Agilent ChemStation software. Injections were performed at 50 mbar for 6 s and the temperature was 25°C. Silica capillary column with 50 µm inner diameter, 64 cm total length, and 56.5 cm effective length was used for the separations (Polymicro Technologies, Phoenix, AZ, USA). By first rinsing with deionized water for 10 minutes and then 1 mol/ L sodium hydroxide (NaOH) during 30 minutes, the condition of the fused silica capillary was assessed. The capillary was rinsed with buffer solution for 10 minutes, deionized water for 10 minutes, and 0.1 mol/ L NaOH for 15 minutes when the work begins. Between each run, the capillary flushed for 2 minutes with 0.1 mol/ L NaOH and deionized water and then for 5 minutes with the running buffer.

The Shimadzu UV-1800 spectrophotometer was used to measure absorbance to specify the total phenolics of each sample. For the determination of DPPH radical scavenging activity, a 96-well microplate reader (BioTek Power Wave XS (USA)) was used.

Gen5 Data Analysis software was used to assess the measurements and computations of the DPPH radical scavenging activity data.

The moisture measurements were performed with an infrared moisture analyzer (Shimadzu MOC63U).

Water purified using a model system (Elga Purelab Option-7-15, UK) was used to prepare all solutions, which were then kept at 4°C.

Samples of pekmez were precisely weighed at 100 mg. After being vortexed for 5 minutes at 3000 rpm to extract each sample into deionized water (5 mL, 80°C), the samples were incubated for 15 minutes in a water bath. For 10 minutes, the mixtures were centrifuged at 3500 rpm. Before injecting the capillary column to measure the contents of sugar and organic acids in pekmez samples, the supernatant was filtered via microfilters with 0.45 µm pore sizes.

Each pekmez sample was carefully weighed at 100 mg before being extracted with a 5 mL solution of methanol: water (80:20, v/v) by vortexing for five minutes at 3000 rpm and sonicating for fifteen minutes. The mixture was centrifuged at 3500 rpm for 10 min. Before analysis, the supernatant was filtered using microfilters with 0.45 m pore sizes. The total phenolic content and DPPH radical scavenging capacity of the pekmez samples were assessed using this extract.

The analysis of glucose and fructose in pekmez was performed using a modified CE method developed by Gürel et al. (2006). This method was also successfully applied to Anatolian honey (Kaygusuz et al., 2016) and honey bee-pollen samples (Kalaycıoğlu et al., 2017), melon cultivars (Kolaylı et al., 2016), and pomegranate juices (Tezcan et al., 2009) by our group in previous studies.

Glyclglycine which is a dipeptide was used as the separation buffer in this method. Fructose and glucose were separated from each other and detected indirectly without any need for derivatization and a buffer additive. In order to obtain an optimal separation, glycylglycine at 50 mmol/L (pH: 12.52) was used. The sample injection was performed at 50 mbar for 6 s at 25 kV. A reference wavelength of 207 nm was used to establish the wavelength at 350 nm.

The amounts of glucose and fructose in each pekmez sample were calculated using calibration curves. For both sugars, the calibration curves were constructed with a correlation coefficient of 0.996 between 2 mmol/L and 8 mmol/L. The limit of detections (LODs) for glucose and fructose were 25.2 mg/mL and 23.1 mg/mL, respectively. Recovery percentages for glucose and fructose were between 93.2 and 107%.

The capillary electrophoresis method, which has previously been used to analyze many foods and beverages, was used to assess the organic acids in pekmez samples (Tezcan et al., 2009; Kaygusuz et al., 2016; Kolaylı et al., 2016; Kalaycıoğlu et al., 2017).

In this method, 2,6-pyridinedicarboxylic acid (PDC) which is a chromophore, was used as a separation buffer. The separation buffer was supplemented with a cationic surfactant, CTAB, to facilitate the rapid coelectroosmotic migration of organic acids. For the best separation, the buffer was chosen to be 5 mmol/L PDC + 0.1 mmol/L CTAB (pH: 5.60). The injections were carried out from the negative side for 6 s at 50 mbar. The running voltage was set at -25 kV. With a reference wavelength of 200 nm, the detection wavelength was 350 nm.

The calibration curves for all organic acids were constructed between 0.250-4.50 µg/mL and the correlation coefficients were changed between 0.992 and 0.999. Calibration curve equations were used for the calculation of organic acid levels in the pekmez samples.

The Folin-Ciocalteu (FC) method (Singleton and Rossi, 1965), which is generally preferred in the calculation of the total phenolic content was used in this study. 7.5 g /100 mL sodium carbonate solution and 1:10 diluted Folin-Ciocalteu's reagent were prepared. Gallic acid (GA) was used as a reference chemical standard.

The stock solution of GA (500 µg/mL) was prepared in distilled water. Three hundred µL of GA in different concentrations were blended with FC reagent (1500 µL) and sodium carbonate solution (1200 µL). The resulting concentrations of GA were between 35-280 µg/mL. For a stable hue, each mixture was left to stand at room temperature for 10 minutes. At 760 nm, the absorbances of various solutions were recorded. The calibration equation for GA was $y = 0.0036x + 0.1445$ ($r^2 = 0.9996$).

The samples were prepared by following the procedure as described for the gallic acid standard above and the absorbances were measured at 760 nm. The total phenolic contents were calculated using the calibration equation of gallic acid. Results were given in terms of mg/g of gallic acid equivalents (GAE).

Each pekmez sample's capacity to scavenge free radicals was assessed using the mostly unaltered DPPH assay developed by Blois (1958). DPPH absorbs at 517 nm in its radical form. In the presence of antioxidant species, its absorption decreases due to the reduction. In this study, 10 µL of pekmez extracts at different ratios (w/v) were added into a 90 µL methanolic 0.1 mmol/L DPPH solution. The mixtures were let to stand for 30 minutes at 25°C, after which the absorbance at 517 nm was measured. The percentage inhibition of DPPH used to represent the results. Equation 1 was used in order to calculate the % inhibition:

$$\text{Percentage inhibition (I\%)} = [(Ac - As)/Ac] \cdot 100 \quad (1)$$

As : Absorbance of the samples

Ac : Absorbance of the control

Moisture contents of the pekmez samples were measured by the loss on drying technique using a moisture analyzer. The samples were weighed on moisture balance and superheated until the end of the drying period by infrared. The temperature was set at 105°C. All the samples reached equilibrium at 10 min. The results were recorded as %moisture (w/w).

RESULTS AND DISCUSSION

Sugars

The sugars of pekmez which make it sweet and flavorful are due to its fruits. These sugars include disaccharides like sucrose and monosaccharides like fructose and glucose. Most fruits have a mixture of these different sugars.

Table 1 displays the fructose and glucose concentrations of the pekmez samples. Glucose amounts are found be between 22.6 – 35.6 g/100 g and fructose levels changes from 20.1 g/100 g to 35.8 g/100 g. Fructose/Glucose (F/G) ratios varied between 0.89 and 1.12. The different types of fruit could be the cause of the vast range of fructose/glucose ratios in the pekmez samples. The F/G ratios of raw fruits of the related pekmez samples were searched from the literature. The F/G ratio of the blueberry (0.95) (Ayaz et al., 2000), pomegranate (0.57) (Legua et al., 2012), and grapefruit (1.02) (Orak et al., 2009) were found to be lower than those of pekmez samples produced from these fruits. The numbers in the brackets are the average F/G ratios. The average F/G ratio of juniper fruits (1.63) from Anatolia (Türkoğlu et al., 2008) was higher than that of juniper pekmez (0.94). Geçer et al. (2016) reported the fructose and glucose contents of 40 accessions of black mulberry fruits from Anatolia and the F/G ratios were calculated between 0.64 – 1.27. Dikkaya et al. analyzed the sugar content of 90 Anatolian carob genotypes and the F/G ratios were between 0.74 – 1.25. In the study of Forney et al. (2012), glucose in cranberry fruit was between 74 and 82% of the total sugars whereas fructose was between 16 to 24% which means the F/G ratios were under 1.00.

Pomegranate, black mulberry, grape, and cranberry pekmez include more or less the same amount of sugars. Glucose and fructose amounts of carob, juniper, and blueberry pekmez have lower than those of them. Figure 1 shows the electropherogram of the grape pekmez sample.

Several pekmez samples' total sugar and individual sugar contents were reported using various methods. The sugar contents of different grape pekmez were determined by HPLC (Turkben et al., 2016). Fructose amounts were found to be between 22.34 – 34.69 g/100 g whereas glucose ranged from 27.57 to 41.11 g/100 g. The mean values of fructose and glucose amount in 4 different kinds of pekmez samples were reported between 10.83 – 31.75 g/100 g and between 11.98 – 32.38 g/100 g, respectively (Şimşek and Artik, 2002). Total sugar contents of 10 mulberry pekmez were reported between 64.04 – 71.40% (Salık et al., 2021). Aliyazıcıoğlu et al. (2009) analyzed glucose and sucrose by a spectrophotometric technique. Glucose amounts were ranged from 41.0 and 66.0 g/100 g in four different kinds of pekmez. Alaşalvar et al. (2005) measured the sugar levels of cherry pekmez by HPLC. Fructose (13.76 g/100 g) and glucose (16.39 g/100 g) were predominant components among the other sugars such as xylose (0.48 g/100 g), arabinose (0.71 g/100 g), sorbitol (7.43 g/100 g), and sucrose (0.05 g/100 g).

Organic acids

Organic acids improve the physicochemical and sensory properties of food products. Moreover, they are commonly used as food preservatives and exhibit antimicrobial activity against food pathogens. Figure 2 illustrates an example electropherogram of a pekmez sample (grape). Pekmez extracts were further diluted to quantify gluconic acid as shown in the on-set electropherogram. Table 2 lists the organic acid concentrations in the pekmez samples.

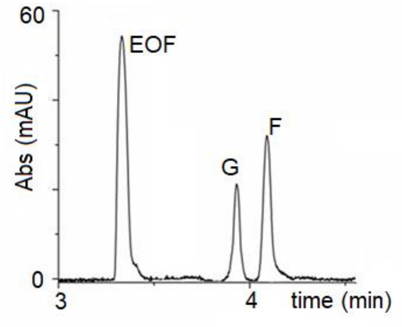

Figure 1. Electropherogram of 1:10 diluted grape pekmez sample (G: Glucose F: Fructose)

Table 1. Glucose, fructose contents and F/G rates of pekmez samples

Pekmez Samples	Glucose (g/100 g ± SD)	Fructose (g/100 g ± SD)	F/G
Carob	22.6 ± 1.2	20.1 ± 3.9	0.89
Juniper	23.4 ± 2.9	21.9 ± 2.4	0.94
Blueberry	25.8 ± 2.7	26.9 ± 4.3	1.04
Pomegranate	32.9 ± 3.9	33.4 ± 4.1	1.02
Black mulberry	35.6 ± 2.5	34.7 ± 2.8	0.97
Grape	32.0 ± 3.1	35.8 ± 4.2	1.12
Cranberry	32.4 ± 1.8	33.9 ± 4.1	1.05

Oxalic acid, tartaric acid, malic acid, citric acid, succinic acid, acetic acid, lactic acid, and gluconic acid are all present in varying concentrations in all of the pekmez samples. Higher organic acid levels were obtained in the grape and black mulberry pekmez among all samples. The major organic acid is gluconic acid (6.7 – 16 g/kg) in all pekmez samples. As far as is known, there has not been any investigation of the precise organic acid composition of pekmez.

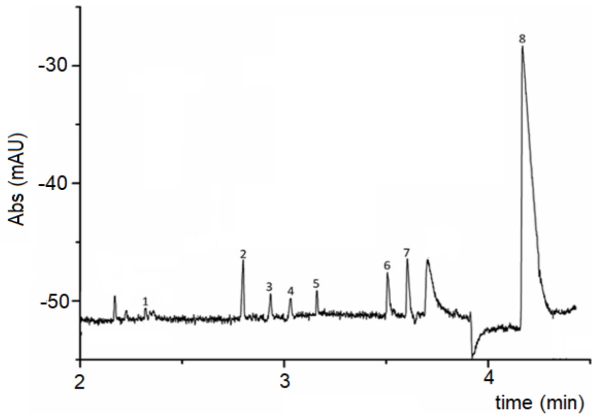

Figure 2. Electropherogram of grape pekmez extract. (1)Oxalic acid, (2)Tartaric acid, (3)Malic acid, (4)Citric acid, (5)Succinic acid, (6)Acetic acid, (7)Lactic acid, (8)Gluconic Acid. On-set electropherogram shows the analysis of the diluted pekmez extract at quantification of gluconic acid

Table 2. Organic acids in pekmez samples

Pekmez Samples	Oxalic Acid	Tartaric Acid	Malic Acid	Citric Acid	Succinic Acid	Acetic Acid
Carob	0.012 ± 0.03	0.18 ± 0.05	0.32 ± 0.02	0.27 ± 0.03	0.25 ± 0.03	0.15 ± 0.03
Juniper	0.012 ± 0.01	0.21 ± 0.03	0.39 ± 0.02	0.35 ± 0.03	0.23 ± 0.04	0.14 ± 0.04
Blueberry	0.072 ± 0.012	0.19 ± 0.03	0.38 ± 0.03	0.35 ± 0.03	0.095 ± 0.012	0.087 ± 0.012
Pomegranate	0.065 ± 0.017	0.22 ± 0.03	0.31 ± 0.01	0.28 ± 0.02	0.18 ± 0.02	0.11 ± 0.04
Black mulberry	0.11 ± 0.01	0.25 ± 0.02	0.35 ± 0.03	0.23 ± 0.03	0.21 ± 0.02	0.12 ± 0.01
Grape	0.083 ± 0.001	0.32 ± 0.01	0.43 ± 0.03	0.41 ± 0.04	0.29 ± 0.05	0.16 ± 0.02
Cranberry	0.062 ± 0.022	0.23 ± 0.03	0.39 ± 0.03	0.36 ± 0.03	0.24 ± 0.04	0.13 ± 0.03

Table 2. Organic acids in pekmez samples (continued)

Pekmez Samples	Lactic Acid	Gluconic Acid
Carob	0.98 ± 0.11	10.0 ± 2.3
Juniper	1.1 ± 0.3	9.0 ± 1.6
Blueberry	0.7 ± 0.1	6.7 ± 2.8
Pomegranate	1.3 ± 0.1	8.4 ± 4.1
Black mulberry	0.86 ± 0.12	15 ± 4
Grape	1.2 ± 0.1	18 ± 3
Cranberry	0.9 ± 0.1	7.2 ± 2.3

The results are presented as means with standard deviations (n = 3), g/kg.

Total phenolics and antioxidant activity

One of the main contributors to the antioxidant activity of dietary products is thought to be phenolic chemicals. Table 3 lists the total phenolics in the pekmez samples. As seen from the table, total phenolic contents were ranged from 265.3 to 1071 mg GAE/g. The highest value was observed in grape pekmez whereas blueberry pekmez has the lowest total phenolic content.

In the literature, there are some studies reporting the total phenolics of pekmez. Total phenolics of mulberry pekmez samples from Erzincan (n=10) was evaluated and found to be between 3.92-10.05 mg GAE/g (Salık et al., 2021). The total phenolic content of grape, date, apricot, mulberry, and carob pekmez was changed between 138-243 mg GAE/g (Aliyazicioglu et al., 2009). Aydinlik and Battaloglu (2016) analyzed 50 grape pekmez samples for their phenolic contents and gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid and ferulic acid were found. The mean (n=50) total phenolic content was reported as 3359.25 mg GAE/g dry weight (Kaya and Belibağlı, 2002). Turkben et al. (2016) determined caffeic acid, ellagic acid, ferulic acid, gallic acid, p-coumaric acid, and rutin hydrate in 14 different grape pekmez samples.

The information about the polyphenol compound spectrum in different pekmez samples is missing. However, it could be expected that pekmez contains similar polyphenols to fruits in which it is produced from. Haminiuk et al. (2012) compared the total phenolic contents of fruit in their review article. Grape was reported as one of the richest phenolic substance sources in comparison to other fruits such as berries.

Antioxidants are vital nutrients with the power to shield the body from the harm brought on by oxidative stress brought on by free radicals. Based on their ability to scavenge DPPH radicals, the pekmez samples' antioxidant potential was examined. It is a commonly used test for figuring out how effective antioxidants are in substances and natural products. The rate of DPPH discolouration was used to analyze the antioxidant substance's capacity to scavenge DPPH radicals. Sample to solution ratios was optimized as ¼ (w/v) and ½ (w/v) to screen DPPH radical scavenging activities of the pekmez samples besides without dilution. Figure 3 shows the average inhibition (%) values against the sample to solution ratios of the samples. Carob pekmez had the highest DPPH radical scavenging activity, with an inhibition value of 96.28%. Juniper and pomegranate pekmez followed carob pekmez, respectively. Cranberry pekmez was the least active pekmez, however, it inhibited the DPPH by more than %50.

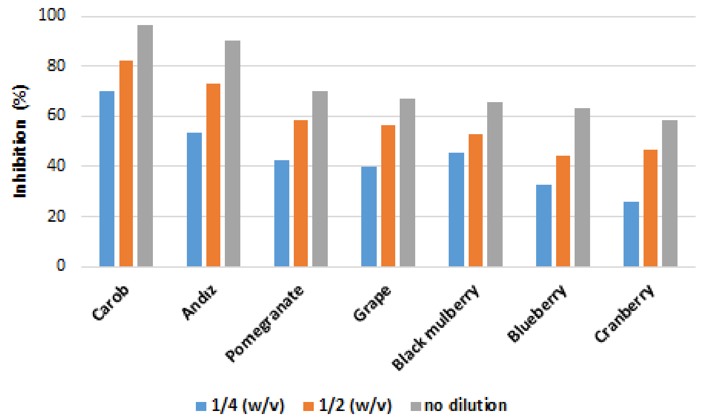

Figure 3. DPPH % inhibition values of the pekmez sample

Moisture content

The moisture content of a food product has a significant impact on the physical properties such as texture, appearance, products' taste, weight, and density. The food products' shelf life, food quality measurements, and even legal requirements are related to moisture contents.

The moisture contents of pekmez samples in terms of %w/w are given in Table 3. The cranberry and grape pekmez have the highest moisture values, as shown in the table. There is a significant difference between these two pekmez samples and the other samples. The other samples have moderate the same moisture content.

Table 3. Total phenolic and moisture contents in the pekmez samples

Pekmez Samples	Total phenolic contents (mg GAE/g ± SD)	Moisture contents (w/w ± SD)
Carob	598 ± 9	5.10 ± 0.04
Juniper	793 ± 12	2.41 ± 0.01
Blueberry	265 ± 5	3.27 ± 0.01
Pomegranate	320 ± 4	3.27 ± 0.01
Black mulberry	376 ± 7	4.32 ± 0.03
Grape	1070 ± 15	12.24 ± 0.17
Cranberry	431 ± 7	19.06 ± 0.34

The results are presented as means with standard deviations (n = 3), g/kg

CONCLUSION

In this study, for the determination of sugar and organic acids in different kinds of pekmez, rapid and simple capillary electrophoresis techniques were used. Pekmez samples were evaluated for organic acid contents in this study, for the first time in the literature. When considered the sugar content, the pomegranate, juniper, cranberry, and blueberry pekmez were evaluated for the first time in this study. Additionally, the samples' total phenolic levels, antioxidant activity, and moisture contents were reported. On the basis of the above findings, pekmez seems to be an important source of sugar, organic acid, and antioxidants. The differences observed between the samples may be attributed to the fruits which are produced from.

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Conflict of Interest

The author declared that there is no conflict of interest.

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