RESEARCH ARTICLE

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Phenolic composition of common produced raisins in Türkiye

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Abstract

This study aimed to determine individual phenolic compounds of common produced Turkish raisins. As material, Sultan 7, Antep Karasi and Razaki raisins were used. The HPLC method was performed for the analysis of 11 phenolic compounds. The major phenolic acid was *trans*-caftaric acid and the major flavan-3-ol was also (+)-catechin. The *trans*-caftaric acid varied between 21.56 and 46.84 µg/g in the samples and (+)-catechin between 2.21 and 74.12 µg/g. Caffeic acid was the second most abundant phenolic acid with 11.06-21.54 µg/g. The highest gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid and quercetin hydrate concentrations were found in Razaki, and *trans*-caftaric acid, p-coumaric acid and *trans*-resveratrol in Antep Karasi. The *trans*-resveratrol was only detected in Antep Karasi raisin with 0.84 µg/g. The strong significant correlations were observed between investigated phenolic compounds. As result, the phenolic profiles of three Turkish raisins were revealed, and the correlations among these compounds were investigated. The findings on raisins indicated that Turkish raisins are a good source of polyphenols.

Keywords: Raisin, Polyphenol, Caftaric acid, Resveratrol, Correlation

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INTRODUCTION

Türkiye has suitable ecological conditions for viticulture and a considerable genetic grapevine diversity. Raisins are an important dried product with 290,000 tons produced on average in the last five years in Türkiye (TMO, 2021). In addition, Türkiye is one of the largest raisin (dried grape) producer and exporter in the world. Although both seeded and seedless grapes are dried, mostly seedless raisins are exported. Seeded raisins are generally traded in the domestic market. The grapes are dried by dipping to a solution or spraying them with a solution under the sun in our country. The used dipping solution includes 5% potassium carbonate and 1% dipping oil. This pre-treatment accelerates drying by resolving and removing the wax layer on the grape surface. This process provide increment in the colour lightness depending on the reduction in drying period.

In recent years, consumer demand and interest in healthy and reliable dried fruits have increased. In particular, raisins are one of the most often preferred because of their high nutritional characteristics. Raisins are a rich source of phenolic compounds. They contain remarkable concentration of flavonol glycosides and phenolic acids (Karadeniz et al., 2000). Previous studies have showed that raisins contain gallic, *trans*-caftaric, *trans*-coutaric, coumaric, protocatechuic and ferulic acids, *trans*-resveratrol, catechin, epicatechin, rutin, myricetin, quercetin, kaempferol, malvidin-3-O-glucosides and its acylated esters (Karadeniz et al., 2000; Breksa, et al., 2010; Kelebek et al., 2013). Polyphenols are generally classified into two groups as flavonoids and non-flavonoids. In grape pulp, phenolic acids form a group of non-flavonoids. Grape-based products can be unstable due to phenolic acids, which affect the constitution of colour pigments such as yellow or brown. This is one of the most prominent problems associated with the drying of white grapes. (Kelebek et al., 2013). Flavonoid group consist of flavan-3-ols, flavonols, and anthocyanin. Flavonoids having high antioxidant power, have several functional properties for human health (Kelebek et al., 2013; Guler et al., 2022). After the drying process, many of the grape polyphenols have high bioavailability. According to Schuster et al. (2017), consumption of the raisin in a daily diet reduces blood sugar and pressure, and cholesterol (low density lipoprotein) compared with snacks having equal caloric carbohydrates. In addition, raisin consumption is associated with the reduction of cardiovascular diseases and also positively affects intestinal flora. Beside, raisin play role in the prevention of many chronic diseases such as cardiovascular diseases, type 2 diabetes, intestinal diseases and dental caries (Schuster et al., 2017). As similar, Williamson and Carughi (2010) reported that raisins can reduce the postprandial insulin response, control glycemic index, affect certain oxidative biomarkers, and promote satiety.

There are limited studies of raisin phenolic composition although Türkiye is one of the most important raisin producer and exporter in the world. The current study aims to determine individual phenolic compounds of Sultan 7 that is registered a few years ago, Antep Karasi and Razaki raisins.

MATERIALS AND METHODS

Materials

The main materials of this study are Sultan 7, Razaki and Antep Karasi raisins that were dried under the sun. The fresh grapes were supplied from the vineyards of Viticulture Research Institute, Manisa. After being dried, raisins were picked up from drying area and stored at +4 °C until used for analysis. During the drying process, the weather conditions were at seasonal normals and no rain. Drying times were 7 days for Sultan 7 and 10 days for Razaki and Antep Karasi. Figure 1 shows the images of the analysed raisins. Sultan 7 a seedless grape variety and registered by Viticulture Research Institute, Manisa in 2011. This grape variety is commonly used for drying because of high drying efficiency and raisin quality. Razaki is a seed grape variety and it is used for table and drying. Its berries are green-pinky yellow, long ellipsoidal, large (6-7 g), 2-4 seed and neutral flavor. The clusters of Razaki are winged conical-cylindirical, large (400-500 g) and loose (Çelik, 2002). Antep Karasi is synonyms of Kilis karasi and Horoz karasi grape varieties. Its berries are blue-black, long ellipsoidal, very large (8-9 g), 2-3 seed and tanninous, and clusters are winged conical, large (700-800 g) and well-filled (Çelik, 2002). It is mostly grown for table and drying purposes.

Figure 1. Raisin pictures (Sultan 7, Razaki and Antep Karasi)

Sample preparation and extraction

Extraction of the polyphenols from raisin samples was performed using the procedure described Kelebek et al. (2013) with slightly modifications. Raisin samples were powdered in liquid nitrogen by using a homogenizer (Ultra Turrax T25 D, Ika, Germany) at 10,000 rpm. Obtained raisin powder mixed with 1 volume of acetone and then homogenised by using Ultra Turrax for 3 min at 10,000 rpm. Homogenised samples were filtered using a Buhner funnel Whatmann no.1. The extraction procedure was repeated with aqueous methanol (30:70 v/v) to till a clear solution was achieved. The obtained filtrate was centrifuged for 10 min at 4000 rpm (Nuve NF 400, Türkiye) and the clear part was separated. The methanol was evaporated using by a rotary evaporator (Ika RV 10, Germany) under vacuum conditions at 40 °C. The solid phase extraction (SPE) was used for the purification of obtained extract. Before the analysis, the preconditioning of the SPE cartridge C18 (Bond Elut C18, 100mg 3 mL, Agilent Technologies, US) was conducted with 5 mL ethyl acetate, 5 mL methanol (0.01% HCl) and 2 mL distilled water (0.01% HCl), respectively. The 1 mL extract was added to the preconditioned cartridge followed by 2 mL distilled water. Then, the cartridge was dried by using a block heater (TAB 24-2, Türkiye) under nitrogen gases at 35 °C. The phenolic compounds were loaded with ethyl acetate (5 mL) and evaporated under reduced pressure at 40°C in the rotary evaporator. The purified extract was dissolved at methanol.

Analysis of phenolic compounds by HPLC

The method reported by Özkan and Göktürk Baydar (2006) with some modifications were performed to quantify the individual polyphenols. The HPLC (high performance liquid chromatography) system was an Agilent Technologies 1260 Infinity equipped with a quaternary pump, on-line degasser, column heater, auto-sampler, and UV-diode array detector (Agilent, Waldborn, Germany). The analytical separation was performed using by C18 ODS 250×4.6 mm, 5 µm (Agilent) column. The following phenolic compounds were detected: Gallic acid, (+)-catechin, caffeic acid and p-coumaric acid at 280 nm, *trans*-caftaric acid, vanillic acid, (-)-epicatechin, ferulic acid, sinapic acid and *trans*-resveratrol at 320 nm, and quercetin hydrate at 360 nm wavelength. The samples were filtered by using a syringe filter (PTFE, 0.45 µm, Sartorius) before injection. The flow rate was set to 1 ml/min and the column temperature to 30 °C. The filtered sample was injected into the system as 10 µl volume. The ultrapure water: formic acid (99.8:0.2 v/v) (A) and methanol (B) were mobile phases. The gradient program started with 100%A and changed to 5% B along 3 min, and 20% B along min 18, held for 20% B 18 min (isocratic step). Followed by elution program was 75% A and 25% B at min 30, 70% A and 30% B at min 40, 60% A and 40% B at min 50, 50% A and 50% B at min 55, and 100% B at min 65. 100% A elution was performed for 5 min to return to the initial condition. The obtained data were analysed using by a software program (Agilent ChemStation OpenLAB). Phenolic compounds were identified according to their retention times and spectra in comparison with analytical standards. The concentration of individual polyphenols was calculated using by calibration curves. The results were expressed as µg in g raisin. The retention times, wavelength, linear range, equation and correlation coefficient of investigated phenolic compounds were presented in Table 1.

Statistical analysis

All analysis were performed in triplicate (n=6), and obtained means are reported with the standard deviations. Results were analysed by one-way ANOVA test, Duncan's multiple range test was used to compare the significant differences of the mean values at 0.05 level. In addition, the correlation coefficients of Pearson were calculated to examine the relationships among polyphenols.

RESULTS AND DISCUSSION

The results for polyphenol compounds quantified in raisin samples are presented in Table 2. The statistical differences were found between raisin samples for gallic acid, *trans*-caftaric acid, (-)-epicatechin, *p*-coumaric acid, ferulic acid, sinapic acid, *trans*-resveratrol and quercetin hydrate at level 0.05. However, there were no significant statistical differences among raisins for vanillic and caffeic acid means (p>0.05).

The most abundant phenolic compounds were phenolic acids in raisin samples. The major phenolic acid was *trans*-caftaric acid with amounts of 21.56-46.84 µg/g in the samples. The highest *trans*-caftaric acid content was in Antep Karasi, and the lowest in Sultan 7. The finding of *trans*-caftaric acid concentration in Sultan 7 raisin was higher than previously reported Sultaniye raisin data by Kelebek et al. (2013), but lower than Thompson seedless raisin values obtained by other authors (Karadeniz et al., 2000; Parker et al., 2007; Breksa et al., 2010; Fabani et al., 2017). In a previous study, the *trans*-caftaric acid concentration of Antep Karasi was found as 92.99 mg/kg, and between 20.48 and 114 mg/kg in other four raisin samples (Kelebek et al. 2013). This study *trans*-caftaric acid finding in Antep Karasi was lower than previous reported data. In addition, Fabani et al. (2017) studied on alterations of phenolic compounds in grapes during the drying processing and expressed that *trans*-caftaric acid values in the raisins varied from 3.26 to 19.0 mg/100 g DW. Breksa et al. (2010) found *trans*-caftaric acid concentration ranging from 153.5 to 598.7 mg/kg DW in 16 raisin cultivars. In another study, Karadeniz et al. (2000) reported that *trans*-caftaric acid was 39.6 mg/kg in sun dried raisins, 45.2 mg/kg in dipped raisins and 84.3 mg/kg golden raisins. As similar, Parker et al. (2007) also ex-

Table 1. Linearity parameters of investigated phenolic compounds

Compounds	Retention time, (min)	Wavelength, (λ)	Linear range (µg/g)	Equation	Correlation Coefficient (R^2)
Gallic acid	12.8	280	1-50	y=22.4601x-10.567	0.9999
trans-caftaric acid	25.4	320	1-50	y=25.3824x-16.225	0.9999
Vanillic acid	34.0	280	1-50	y=53.2441x-21.866	0.9998
Caffeic acid	35.4	320	5-50	y=6.2370x-13.5031	0.9979
p-coumaric acid	46.9	280	1-50	y=12.2091x-8.7214	0.9996
Ferulic acid	48.9	320	0.5-25	y=57.2881x-9.1455	0.9999
Sinapic acid	50.1	320	1-50	y=46.1044x-10.5248	0.9999
(+)-Catechin	27.0	280	1-50	y=7.1567x-2.0443	0.9996
(-)-Epicatechin	45.5	320	0.5-25	y=68.0424x-10.1415	0.9999
trans-resveratrol	58.3	320	0.5-25	y=61.0464x-14.638	0.9999
Quercetin hydrate	62.4	360	5-50	y=25.1789x-66.5442	0.9964

Table 2. Phenolic compositions of raisin samples

Phenolic compound (µg/g)	Sultan 7	Antep Karasi	Razaki
Gallic acid	2.09±0.49[c]	4.42±0.93[b]	7.11±1.28[a]
trans-caftaric acid	24.75±0.01[ab]	46.84±9.84[a]	21.56±3.99[b]
Vanillic acid	1.83±0.01	4.67±1.23	2.85±0.36
Caffeic acid	11.06±0.01	11.85±2.39	21.54±7.19
p-coumaric acid	2.80±0.65[b]	18.76±4.38[a]	10.12±0.44[ab]
Ferulic acid	0.93±0.37	0.68±0.15	2.51±1.40
Sinapic acid	1.74±0.30[b]	1.36±0.24[b]	3.97±0.02[a]
(+)-Catechin	2.21±0.01[c]	17.91±2.86[b]	74.12±8.72[a]
(-)-Epicatechin	0.67±0.02[b]	0.60±0.07[b]	1.23±0.26[a]
trans-resveratrol	nd	0.84±0.09	nd
Quercetin hydrate	7.01±0.33[b]	7.03±0.89[b]	15.82±1.02[a]

The values indicated by different letters within each row are significantly different at 0.05 level.
*nd. Not detected

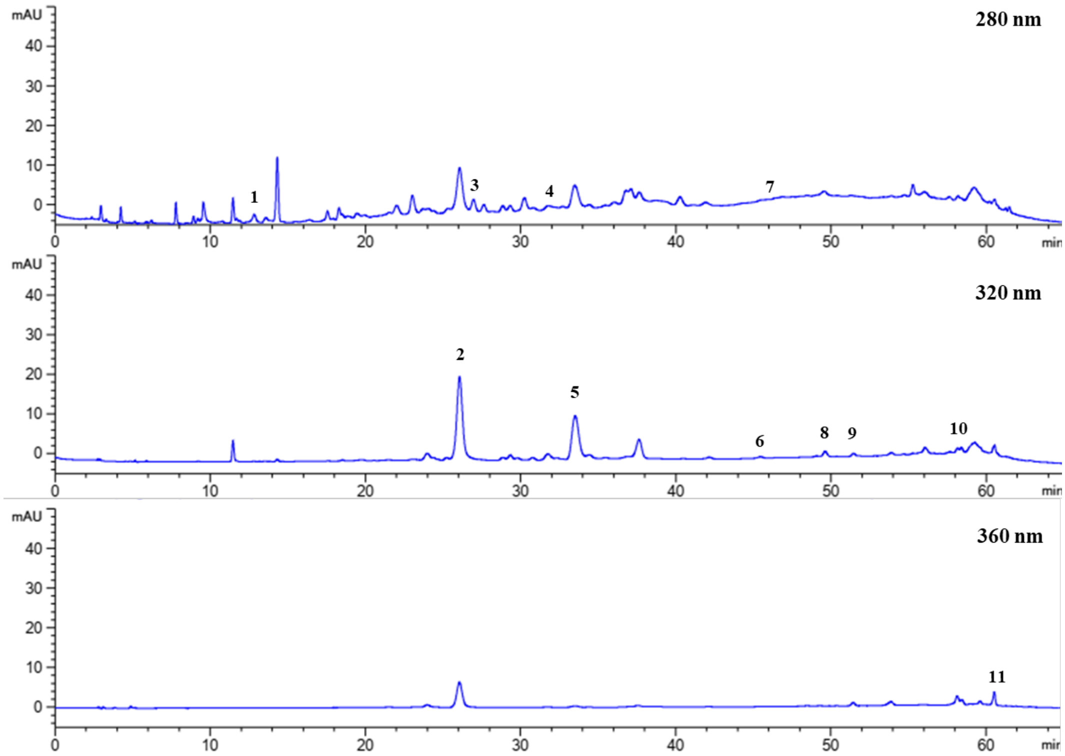

Figure 2. HPLC chromatogram of Antep Karasi phenolic compounds. 1: gallic acid, 2: *trans*-caftaric acid, 3: (+)-catechin, 4: vanillic acid, 5: caffeic acid, 6: (-)-epicatechin, 7: *p*-coumaric acid, 8: ferulic acid, 9: sinapic acid, 10: *trans*-resveratrol, 11: quercetin hydrate

pressed that golden raisin *trans*-caftaric acid (130.4 mg/kg) concentration was higher than sun-dried raisin (41.4 mg/kg).

Gallic acid concentrations were 2.09 µg/g in Sultan 7, 4.42 µg/g in Antep Karasi and 7.11 µg/g in Razaki. In a comparison with the literature, current study findings were lower than results of Kelebek et al. (2013) and Meng et al. (2011), and higher than Fabani et al. (2017) datas. Caffeic acid was the second most abundant phenolic acid in raisin samples with 11.06-21.54 µg/g. These caffeic acid findings are compatible with literature (Meng et al., 2011). Regarding investigated raisins, *p*-coumaric acid concentrations varied between 2.80 and 18.76 µg/g. Meng et al. (2011) studied on Chinese raisin phenolic contents and found 2.38-23.45 µg/g DW *p*-coumaric acid in the 10 raisin samples. The current study *p*-coumaric acid findings were in agreement mentioned research. Vanillic acid concentrations were 1.83 µg/g in Sultan 7, 2.85 µg/g in Razaki and 4.67 µg/g in Antep Karasi raisins. Kelebek et al. (2013) reported that vanillic acid means varied from 0.27 to 0.98 µg/g DW in five raisin samples and the highest value was in Antep Karasi. The vanillic acid results were higher than the last mentioned study findings. The highest ferulic acid was in Razaki with 2.51 µg/g, followed by Sultan 7 with 0.93 µg/g and Antep Karasi with 0.68 µg/g. In literature, Kelebek et al. (2013) reported considerably low vanillic acid concentration (0.40-1.49 mg/kg) in five raisins while Meng et al. (2011) found notably high results in Chinese 10 raisins. In a comparison with the literature, our findings closer to Kelebek et al. (2011) datas. Sinapic acid amounts were determined 1.74 µg/g in Sultan 7, 1.36 µg/g in Antep Karasi and 3.97 µg/g in Razaki raisins.

The Antep Karasi HPLC chromatogram is indicated on Figure 2. Considering investigated Sultan 7, Antep Karasi and Razaki raisins, it is revealed that they contains of considerable individual phenolic acids. In particular, *trans*-caftraric, p-coumaric and caffeic acids were abundant in Turkish raisins.

(+)-Catechin and (-)-epicatechin compounds and their polymers are very powerful antioxidants and they have more antioxidant activity than vitamin E (Rice Evans et al., 1997). Their concentration in grape products is considerably significant for this reason. In the present study, (+)-catechin and (-)-epicatechin from flavan-3-ols were investigated in raisin samples. The highest (+)-catechin concentration was found in the Razaki raisin with 74.12 µg/g, followed by Antep Karasi with 17.91 *µ*g/g and Sultan 7 with 2.21 µg/g. In a study, (+)-catechin concentrations of five Turkish sun-dried raisins were detected between 56.31 and 419 mg/kg (Kelebek et al. 2013). Meng et al. (2011) found that (+)-catechin ranged from 10.4 to 66.47 µg/g DW in 10 Chinese raisins. In another study, (+)-catechin concentrations in Argentinean sun-dried raisins were determined 15-158 mg/100 g DW (Fabani et al., 2017). In this respect, our current findings were low compared with reported results by Kelebek et al. (2013) and Fabani et al. (2017), and in accordance with expressed findings by Meng et al. (2011). (-)-Epicatechin contents varied from 0.60 to 1.23 µg/g in raisin samples. In previous studies, (-)-epicatechin concentrations in raisins were reported ranging from 19.18 to 117 mg/kg by Kelebek et al. (2013) and 15-27 mg/100 mg DW by Fabani et al. (2017). In addition, Fabani et al. (2017) could not detected (-)-epicatechin in sun-dried Sultaniye and Superior raisins. Moreover, it is claimed that the drying process under the sun could be completely degraded the flavan-3-ols in raisin because of probably enzymatic oxidation (Karadeniz et al., 2000; Fabani et al., 2017). In particular, our (-)-epicatechin findings in Sultan 7 and Antep Karasi were low, but they were not consistent with results reported by these literatures. It is thought that these differences may be affected by cultivar and drying conditions as well as variety differences. USDA data related to the flavan-3-ols in raisins support our findings (Haytowitz et al., 2018).

Resveratrol is a polyphenol compound classified in stilbenes and has possible beneficial effects on human health with high antioxidant properties. *Trans*-resveratrol was only detected in Antep Karasi raisin (0.84 µg/g). Breksa et al. (2010) detected *trans*-resveratrol in the justly one raisin sample (B53-122) with 0.8 µg/g DW in investigation of 16 raisins. Karadeniz et al. (2000) could no detected the *trans*-resveratrol in Thompson Seedless raisin, and explained this situation by the fact that the capability of grapes to produce resveratrol is lost during the ripening period. On the other hand, Roychev et al. (2020) reported that *trans*-resveratrol concentrations were ranged from 1.97 to 18.62 mg/kg in 26 raisins of seedless hybrids, Corinthian Black raisin and Gamay Freaux and Sangiovese grapes. This current study result of *trans*-resveratrol was in agreement with the literature, since its concentration could change depending on variety and fungal infections (Karadeniz et al., 2000; Fabani et al., 2017; Roychev et al., 2020).

Table 3. The correlations among phenolic compounds in raisins

Correlation	**Gallic acid**	**trans-caftaric acid**	**(+)-Catechin**	**Vanillic acid**	**Caffeic acid**	**(-)-Epicatechin**	**p-coumaric acid**	**Ferulic acid**	**Sinapic acid**	**Quercetin hydrate**
Gallic acid	1	0.088	0.901**	-0.043	0.584	0.784**	0.462	0.458	0.715*	0.788**
trans-caftaric acid	0.088	1	-0.271	-0.006	-0.409	-0.318	0.821*	-0.360	-0.349	-0.595
(+)-Catechin	.901**	-0.271	1	0.145	0.713	0.874**	0.127	0.629	0.856*	0.939**
Vanillic acid	-0.043	-0.006	0.145	1	0.156	-0.254	0.099	-0.129	-0.407	0.108
Caffeic acid	0.584	-0.409	0.713	0.156	1	0.657*	-0.414	0.673	0.532	0.714*
(-)-Epicatechin	0.784**	-0.318	0.874**	-0.254	0.657*	1	-0.069	0.852**	0.852**	0.883**
p-coumaric acid	0.462	0.821*	0.127	0.099	-0.414	-0.069	1	-0.203	0.002	-0.129
Ferulic acid	0.458	-0.360	0.629	-0.129	0.673	0.852**	-0.203	1	0.730*	0.673
Sinapic acid	0.715*	-0.349	0.856*	-0.407	0.532	0.852**	0.002	0.730*	1	0.912**
Quercetin hydrate	0.788**	-0.595	0.939**	0.108	0.714*	0.883**	-0.129	0.673	0.912**	1

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

Quercetin is a polyphenol that belongs to the flavonol group. Potential benefits of quercetin are protection from oxidative stress, cardiovascular disease, cancer, anti-inflammatory and age-related neurological degeneration (Carughi et al., 2008). Quercetin hydrate varied between 7.01 and 15.82 µg/g in raisin samples. The highest quercetin concentration was in Razaki, and the lowest in Sultan 7 raisin. Breksa et al. (2010) reported that quercetin 3-*o*-glucoside contents changed from 7.4 to 69.3 µg/g DW in raisin samples. Chinese raisin quercetin concentrations varied between 17.95 and 326.7 µg/g DW (Meng et al., 2011). The other study showed that the quercetin 3-*o*-glucoside concentrations of Turkish raisins were detected from 2.79 to 12.83 mg/kg (Kelebek et al., 2013). Karadeniz et al. (2000) found that quercetin glycoside were 7.3-41.5 mg/kg in different processed raisins. The highest quercetin concentration was observed in golden raisins followed by dipped and sun-dried raisins. Findings related to the quercetin were in agreement with mentioned previous studies. It has been revealed that sun exposure to grapes affects the quercetin concentrations (Price et al., 1995). According to Soleas et al. (1997), the grape variety and water status could affect the quercetin concentration.

Pearson correlations between investigated polyphenols are presented in Table 3. The linear correlation coefficients among analysed polyphenols were significant at 0.01 and 0.05 levels. There were strong correlations between gallic acid and (+)-catechin, (-)-epicatechin and quercetin hydrate. Similarly, positive strong correlations were observed between (+)-catechin and (-)-epicatechin and quercetin hydrate. Congruently, significant correlations were also observed between (-)-epicatechin and (+)-catechin, ferulic acid, sinapic acid and quercetin hydrate. In addition quercetin hydrate strongly correlated with gallic acid, (+)-catechin, (-)-epicatechin and sinapic acid at 0.01 level.

CONCLUSION

In this study, the polyphenol profiles of three raisins from Türkiye, which are commonly produced and marketed, were evaluated. The phenolic content findings in raisins indicated that Turkish raisins are a good source of polyphenols. It was determined that Antep Karasi and Razaki being seeded grapes have rather phenolic content than Sultan 7. The most abundant phenolic compounds were phenolic acids in investigated samples**.** The major phenolic acid *was trans*-caftaric acid with 21.56-46.84 µg/g, and the major flava-3-ol was (+)-catechin with 2.21-74.12 µg/g. *Trans*-resveratrol could be only detected in Antep Karasi raisin. The highest gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid and quercetin hydrate concentrations were detected in Razaki, and *trans*-caftaric acid, *p*-coumaric acid and *trans*-resveratrol in Antep Karasi. Further research is needed to determine the phenolic profile of Turkish raisin genotypes and the effects of the drying process.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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