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Farklı Çözücülerle Hazırlanan Bazı Meyve Ekstraktlarının Antioksidan Potansiyeli

Deniz GÜNAL KÖROĞLU¹, Gezzemhan SÜYÜNÇ¹, Rabia YILDIRIM¹, Semra TURAN^{1*}

ÖZET: Bu çalışmada çilek, ahududu, vişne ve kıvılcıkta ekstraksiyon çözücüsü olarak metanol, etanol, %80'lik metanol ve %80'lik etanol kullanılarak fenolik ekstraktlar elde edilmiştir. Herbir meyve ekstraktının toplam fenolik madde miktarı ve farklı konsantrasyonlarda (0.5, 1, 2 and 3 mg ml⁻¹) antioksidan aktiviteleri (demir iyonları indirgeme gücü, linoleik asit emülsiyonunda antioksidan aktivite ve DPPH radikallerini yakalama gücü) belirlenmiştir. Tüm ekstraktların antioksidan aktiviteleri konsantrasyon arttıkça artmıştır. Toplam fenolik madde miktarı ile ekstraktların antioksidan aktiviteleri arasında bir korelasyon bulunmaktadır. Toplam fenolik madde miktarına ve antioksidan aktivite analiz sonuçlarına göre çilek ekstraktları diğer meyve ekstraktlarına kıyasla daha yüksek antioksidan aktiviteye sahip olmuştur ($p<0.05$). Ahududu ekstraktının demir indirgeme gücü en düşük olup, aynı çözücü için vişne ekstraktında kıvılcık ekstraktından daha yüksek indirgeme gücü saptanmıştır. Linoleik asit emülsiyonunda meyve ekstraktlarının tüm konsantrasyonlarında sulu etanol ekstraktları daha yüksek antioksidan aktiviteye sahip olmuştur. Çilek ekstraktları dışında, ahududu %80 metanol ekstraktı en yüksek DPPH radikal yakalama gücüne sahip olup, onu kıvılcık ve vişne ekstraktları takip etmiştir.

Anahtar Kelimeler: Çilek, ahududu, vişne, kıvılcık, antioksidan aktivite

Antioxidant Potential of Some Fruit Extracts Prepared with Different Solvents

ABSTRACT: In this study, phenolic extracts of strawberry, red raspberry, sour cherry, and cornelian cherry were obtained using methanol, ethanol, 80% methanol, and 80% ethanol as extraction solvents. Total phenolic content and antioxidant activities (ferric reducing power, antioxidant activity in linoleic acid emulsion, and DPPH radical scavenging activity) were determined for each fruit extracts at different concentrations (0.5, 1, 2 and 3 mg mL⁻¹). Antioxidant activities of all extracts were increased with increased concentration ($P<0.05$). There was a correlation between total phenol content and antioxidant activity of the extracts. According to total phenolic content and antioxidant activity analyses, strawberry extracts had significantly higher antioxidant activity compared to other fruit extracts ($P<0.05$). Reducing power of raspberry ethanol extract was the lowest and sour cherry had higher reducing power than cornelian cherry for the same solvent. Aqueous ethanol extracts had higher antioxidant activity in linoleic acid emulsion among fruit extracts at all concentrations. Except for strawberry extracts, 80% methanol extract of red raspberry had the highest DPPH radical scavenging activity at all the concentration tested, followed by cornelian cherry and sour cherry extracts.

Keywords: Strawberry, red raspberry, sour cherry, cornelian cherry, antioxidant activity

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INTRODUCTION

Berries, sour cherry (also called tart cherries), and cornelian cherries are special foods with their unique aroma, color, flavor, texture, and chemical content such as minerals, vitamins, antioxidants, and secondary metabolites. Berries and cherries are consumed a lot in their seasons as fresh but their shelf life is very short. Therefore, it is common to consume these fruits as a processed product in the form of dried, frozen, or as jams, jellies, marmalade, syrups, wines, etc. (Cerezo et al., 2010; Bobinaite et al., 2012; Chen et al., 2013a; Mandave et al., 2014). For reasons such as they have a short shelf life or cannot be consumed fresh, they have recently been included in the literature due to their antioxidant properties. They are good sources of antioxidants that have been researched lately (Dragišić Maksimovic et al., 2013). Phenolic compounds in these fruits are flavonoids, especially anthocyanins, phenolic acids, and tannins (Cerezo et al., 2010; Bobinaite et al., 2012). Also, they are rich in anthocyanins, which are responsible for the red color and are powerful antioxidants. Many studies have studied anthocyanin content and antioxidant potential in strawberry (De Souza et al., 2014; Mandave et al., 2014; Chaves et al., 2017; Márquez-López et al., 2020), raspberry (Pantelidis et al., 2007; Çekiç and Özgen, 2010; Chen et al., 2013a; Dragišić Maksimovic et al., 2013; De Souza et al., 2014), sour cherry (Kim et al., 2005; Piccolella et al., 2008; Khoo et al., 2011; Kopjar et al., 2014) and cornelian cherry (Pantelidis et al., 2007; Yilmaz et al., 2009; Hassanpour et al., 2011; Celep et al., 2012; Moldovan et al., 2016). Potential health effects of these fruits associated with anthocyanin were studied in the literature (Ferretti et al., 2010; Chen et al., 2013b; Khoo et al., 2017).

Strawberry, raspberry, sour cherry, and cornelian cherry have a unique phenolic profiles and strong antioxidant activity. The major phenolic compounds in strawberries are ellagic acid, p-coumaric acid, and their esters (Häkkinen et al., 1999; Márquez-López et al., 2020), procyanidins, ellagitannins, (+)-catechin and p-coumaroyl esters (Cerezo et al., 2010). Dominant anthocyanin compounds in strawberry are cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, and pelargonidin-O-rutinoside (Chaves et al., 2017).

Main phenolics in red raspberries are anthocyanins such as cyanidin 3-glucoside and cyanidin-3-rutinoside (Bobinaite et al., 2012; Chen et al., 2013a), flavonoids such as epicatechin (Dragišić Maksimovic et al., 2013), phenolic acids such as ellagic acid (Bobinaite et al., 2012) and tannins such as ellagitannins (Sanghiin H6 and Lambertianin C) (Mullen et al., 2002; Bobinaite et al., 2012). Raspberry also contains a wide variety of quercetin and kaempferol derivatives (Häkkinen et al., 1999; Mullen et al., 2002).

Sour cherry is rich in anthocyanins (cyanidin-3-glucosylrutinoside, cyanidin-3-sophoroside, cyanidin-3-rutinoside and cyanidin-3-glucoside) (Antolovich et al., 2000; Damar and Ekşi, 2012; Wojdyło et al., 2014; Homoki et al., 2016), beside hydroxycinnamates (neochlorogenic acid and p-coumaroylquinic acid), flavonols and flavan-3-ols (catechin, epicatechin, quercetin 3-glucoside, quercetin 3-rutinoside, and kaempferol 3-rutinoside) (Ferretti et al., 2010; Toydemir et al., 2013).

Cornelian cherry (*Cornus mas*) grows wild in Asia and Europe and is cultivated in Turkey being an important producer. Although fresh consumption is not preferred due to the acrid taste, it is consumed as marmalade and jam or in Turkish folk medicine against diabetes and diarrhea (Celep et al., 2012). Cornelian cherry had a high quantity of anthocyanins (cyanidin-3-O-galactoside, pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside, pelargonidin-3-O-glucoside), phenolic acid (ellagic acid, (-) epicatechin) (Moldovan et al., 2016) and flavonoid (quercetin, kaempferol, and aromadendrin 3-O-glycosides) (Popović et al., 2012).

It is difficult to compare the phenolic content and antioxidant activities of extracts from different types of berries in the literature, due to the extracts obtained using different methods (Häkkinen et al., 1999). While many studies determined the phenolic profile of these fruits, there are limited studies that compare their phenolic content and antioxidative activity via different solvent extraction.

The aim of this study is to compare the phenolic content and antioxidant activities of different fruits or their extracts obtained with different solvents. Strawberry (*Fragaria recsa*), red raspberry (*Rubus idaeus*), sour cherry (*Prunus cerasus*), and cornelian cherry (*Cornus mas*) were used in this study and methanol, ethanol, 80% methanol, and 80% ethanol were the extraction solvents. Ferric reducing power, antioxidant activity in linoleic acid emulsion and DPPH radical scavenging activity were methods used to determine antioxidant activity.

MATERIALS and METHODS

Materials

Strawberry (*Fragaria recsa*), raspberry (*Rubus idaeus*), sour cherry (*Prunus cerasus*) and cornelian cherry (*Cornus mas*) were obtained from a local market in Bolu, Turkey. All extracts were stored at -18 °C until the analyses were done. DPPH reagent and linoleic acid (99%) were taken from Sigma-Aldrich (St Louis, USA). Iron (III) chloride hexahydrate was obtained from Acros Organics (New Jersey, USA). Other reagents were obtained from Merck (Darmstadt, Germany).

Preparation of Extracts

Leaves, stems, and seeds of all fruits were removed after cleaning. All fruits were mashed by passing through a kitchen type blender. 40 g of mashed fruits were weighted in a 250 mL flask and 100 mL methanol, ethanol, 80% methanol: water or 80% ethanol: water (v/v) was added to separate flasks. Then, flasks were shaken at 150 rpm using a shaking water bath for 1 h. After waiting overnight at room temperature, all samples were filtered through a filter paper and a second extraction process was performed by adding 100 mL of solvent on the residue. Extracts obtained with the same solvent were combined and filtered through Whatman 1 paper. Alcohol in extracts was evaporated by using a rotary evaporator at 50°C under vacuum. Extracts were transferred into a colored bottle and nitrogen gas was given for 20 min in order to remove the remained alcohol. All extracts were dried using a freeze-dryer and were stored at -18°C. The extraction yield was calculated as g 100 g⁻¹ fresh weight (fw).

Total Phenolic Content (TPC)

TPC of extracts was determined by the Folin-Ciocalteu method according to Iqbal et al. (2008). 0.01 g of extracts were weighted and were dissolved in 5 mL of deionized water. Analyzes were done by taking 0.20 mL of this solution and 0.20 mL deionized water was used for the control sample. A calibration graph was obtained with the absorbance values corresponding to gallic acid at different concentrations (0.01-0.10 mg mL⁻¹). Using the calibration equation, TPC was determined as mg gallic acid equivalent (GAE) per gram of extracts. The analyses were done in triplicate and the results were given in the format of mean ± standard deviation.

Antioxidant Activity of Extracts

Fruit extract solutions were prepared at 0.5, 1, 2 and 3 mg mL⁻¹ concentrations in 50% aqueous (v/v) alcohol from lyophilized extracts. Reducing power and DPPH radical scavenging activity were determined according to the method of Günal and Turan (2018). Conjugated diene method in the linoleic acid emulsion was done according to Günal-Köroğlu et al. (2019). The values of fruit extract solutions were compared to the values of antioxidants such as BHA, BHT, and α -tocopherol at 0.2 mg mL⁻¹

concentration. The results were given as mean \pm standard deviation and analyzes were performed in triplicate.

Statistical Analyses

The statistical analyses were performed with the SPSS package software, version 18.0 (SPSS Inc., Chicago, IL). Results were expressed as means \pm standard deviation of the two or three replicates of each experiment. Analysis of variance was performed. The difference between the mean values was determined in the 95% confidence interval ($P < 0.05$) using ANOVA and Duncan's multiple comparison test.

RESULTS AND DISCUSSION

Extraction Yields

Extraction yields of the strawberry, sour cherry, red raspberry, and cornelian cherry were shown in Table 1. The extraction yields of their extracts were in the range of 5.01% - 12.72%.

Aqueous methanol or ethanol extracts of strawberry and sour cherry had higher extraction yield than their alcohol extracts. Otherwise, the extraction yield of raspberry and cornelian cherry methanol extracts were higher than that of their 80% methanol extracts. However, aqueous ethanol extracts of raspberry and cornelian cherry were higher than their ethanol extracts. Solvent polarity, degree of polymerization of phenols, the interaction between other components affect the solubility of phenolic compounds. Methanol, ethanol, acetone, water, ethyl acetate are generally used for the extraction of phenolic compounds (Naczki and Shahidi, 2004). Anthocyanins, flavonoids are soluble in water, methanol, ethanol, or their acidified solutions (Naczki and Shahidi, 2004; Khoo et al., 2017).

Table 1. Extraction yields and total phenolic contents of extracts

Extract	Solvent	Yield (g 100 g ⁻¹ fresh weight)	Total phenolic content* (mg GAE g ⁻¹ extract)
Strawberry	Methanol	11.76	33.32 \pm 0.71abA
<i>Fragaria vesca</i>	80% Methanol	12.72	33.94 \pm 0.87aA
	Ethanol	8.05	32.11 \pm 0.83bcA
	80% Ethanol	9.35	31.73 \pm 0.61cA
Red Raspberry	Methanol	10.51	17.31 \pm 0.40cC
<i>Rubus idaeus</i>	80% Methanol	8.70	20.31 \pm 0.27bC
	Ethanol	8.31	13.25 \pm 0.47dD
	80% Ethanol	9.35	21.45 \pm 0.79aB
Sour Cherry	Methanol	9.31	18.63 \pm 0.17cB
<i>Prunus cerasus</i>	80% Methanol	12.26	21.56 \pm 0.12cB
	Ethanol	8.71	18.90 \pm 0.57bB
	80% Ethanol	9.35	20.96 \pm 0.12aB
Cornelian cherry	Methanol	8.41	18.02 \pm 0.38aB
<i>Cornus mas</i>	80% Methanol	6.21	16.18 \pm 0.25bD
	Ethanol	5.01	17.69 \pm 0.18aC
	80% Ethanol	7.54	15.37 \pm 0.16cC

*Analyses were done in triplicate and results were given as mean \pm standard deviation. ^{a-d} Small letters show the variation between the extraction solvent of the same fruit ($P < 0.05$). ^{A-D} Capital letters show the variation between fruit extracts of the same solvent ($P < 0.05$).

In this study, each fruit has a different solubility in different solvent systems depending on their phenolic compounds belong to the different groups. It is known that flavonoids, especially anthocyanins, phenolic acids, and tannins are the main phenolic compounds in these fruits (Cerezo et al., 2010;

Bobinaite et al., 2012). Strawberry, raspberry, sour cherry, and cornelian cherry are red colored fruits and anthocyanins are the main phenolic compounds that responsible for their red color (Khoo et al., 2017).

Celep et al. (2012) obtained higher extraction yields (19.69%) for 80% methanolic extract of cornelian cherry than our findings.

Total Phenolic Content

Folin-Ciocalteu assay was used to determine the total phenolic content (TPC) of extracts. It is a colorimetric and indirect method based on oxidation/reduction reactions of molibdotunstate in reagent as a result of the formation of blue color at 760 nm (Singleton et al., 1999). Since the oxygen radical is not used in the method and reduction power of sample is assumed to be equal to the antioxidant capacity. As a result, a fairly good linear correlation is expected between total phenol content and antioxidant activity (Dragišić Maksimovic et al., 2013).

TPC of the strawberry, sour cherry, red raspberry, and cornelian cherry extracts were shown in Table 1. TPC of extracts were in the range of 13.25-33.94 mg GAE g⁻¹ extract. The variation of total phenolic contents of different fruit extracts prepared by the same solvent was found significant (P<0.05). Similarly, the effects of extraction solvent on the total phenolic content of the extracts was significant (P<0.05).

TPC of strawberry extracts among examined fruits was the highest and TPC in all strawberry extracts was close to each other. In addition to this, TPC of 80% methanol extracts was the highest (33.94 mg GAE g⁻¹ extract) among other solvent extracts of strawberry. Márquez-López et al. (2020) reported that methanol extracts of strawberries contained higher total phenolic compounds than ethanol extracts. It is explained by the higher polarity of methanol and the higher solubility of phenolic compounds as the polarity increases, because phenols in extracts are largely related to the polarity of the solvents used (Márquez-López et al., 2020).

TPC of strawberry extracts obtained in this study was found higher than some studies in the literature. When the total amount of phenolic compounds was calculated as mg GAE 100 g⁻¹ fresh weight (fw), mean TPC of strawberry was found as 361.00 mg 100 g⁻¹ fw in this study. Marquez-Lopez et al. (2020) were stated that ethanol and methanol extracts of strawberries were 226.89 and 361.03 mg GAE 100 g⁻¹ fw, respectively. Another study by Mandave et al. (2014) was shown that 0.2% acetic acid and ethanol extracts from two different strawberry types (Camorasa and Sweet Charlie) had 224 and 207.4 mg GAE g⁻¹ fw, respectively. Chaves et al. (2017) emphasized that the highest amount of total phenolic compounds in seven different strawberry samples was 2.48 mg GAE g⁻¹ fw. Mendes et al. (2011) determined the TPC of the strawberry extract was 16.7 mg GAE g⁻¹ extract and was lower than strawberry extracts in this study. De Souza et al. (2014) found that TPC of strawberry extract was 621.92 mg GAE 100 g⁻¹ fw and it was higher than all strawberry extracts in this study.

TPC of raspberry and sour cherry extracts were higher than the cornelian cherry extracts. TPC of aqueous methanol and ethanol extracts of raspberry (20.31 and 21.45 mg GAE g⁻¹ extract) and sour cherry (21.56 and 20.96 mg GAE g⁻¹ extract) were close to each other. TPC in sour cherry or raspberry methanol and ethanol extracts were lower than 80% methanol or 80% ethanol extracts.

It was stated that the TPC was between 214.71 and 619 mg 100 g⁻¹ fw in 15 different raspberry varieties (Chen et al., 2013a); were between 3.72 and 3.24 mg GAE g⁻¹ fw in two different varieties (Dragišić Maksimovic et al., 2013); were ranged from 1486 to 3479 µg g⁻¹ fw in wild and cultivated raspberries (Çekiç and Özgen, 2010) and were between 1052 and 2494 mg GAE 100 g⁻¹ dw in 50% methanol extracts (Pantelidis et al., 2007) in literature. Average TPC of raspberry extracts in this study

was calculated as 167.33 mg GAE 100 g⁻¹ fw and was like those data in the literature. De Souza et al. (2014) identified TPC as 357.83 mg GAE 100 g⁻¹ fw in raspberry. This value was higher than all raspberry extracts in this study.

Low molecular weight phenolic compounds in sour cherry were responsible for highly water-soluble antioxidant activity (Homoki et al., 2016). Kopjar et al. (2014) examined the anthocyanin, flavonoid, and phenolic contents in sour cherry extracts obtained with water, methanol, ethanol, and their acidified solutions. Anthocyanin and phenolic contents were observed high to low in methanol > ethanol > water extracts. Ethanol extract had the highest flavonoid content followed by methanol and water extracts. In other words, methanol was more effective in extracting phenolic substances and anthocyanins, and ethanol was more effective in extracting flavonoids. Methanol acidified with hydrochloric acid was the most efficient extraction solvent for anthocyanin, phenol, and flavonoid. Besides, TPC of methanol, ethanol, and water extracts with 24 hours extraction was 295.75, 285.68, and 186.96 mg GAE kg⁻¹ fw, respectively (Kopjar et al., 2014).

Khoo et al. (2011) prepared samples with water extracts from 34 different sour cherries and determined TPC between 74 and 754 mg GAE 100 g⁻¹ fw. They also emphasized that the total anthocyanin content of sour cherry extracts was quite low, while sour cherry samples had high content of total phenolics. Kim et al. (2005) determined TPC in methanol extracts of four different sour cherries between 146.1 to 312.4 mg GAE 100 g⁻¹ fw. To compare with the data in the literature, the results were also calculated as mg 100 g⁻¹ fw in this study, and average TPC of sour cherry extracts was 199.54. These data showed compatibility with the literature.

Solutions containing aqueous alcohol were more effective in extracting phenolic compounds from sour cherry and raspberry, while the situation was just the opposite for cornelian cherry extracts. Also, ethanol and 80% ethanol extracts of cornelian cherry had lower TPC than methanol and 80% methanol. It was found that TPC of 80% methanol extract of cornelian cherry was 31.25 mg GAE g⁻¹ extract (Celep et al., 2012) and that of 50% methanol extracts were 1592 mg GAE 100 g⁻¹ dw (Pantelidis et al., 2007). It was determined that TPC of cornelian cherries were between 26.59 and 74.83 mg GAE g⁻¹ dw in 12 different species (Yilmaz et al., 2009) and were between 1097.19 and 2695.75 mg GAE 100 g⁻¹ fw in 6 different species (Hassanpour et al., 2011). Also, TPC in acetone extract of cornelian cherry was 489.94 mg GAE 100 g⁻¹ fw in another study by Moldovan et al. (2016). Average TPC of cornelian cherry were 114.14 mg 100 g⁻¹ fw in this study and the lowest average TPC were belong to cornelian cherry among other fruits.

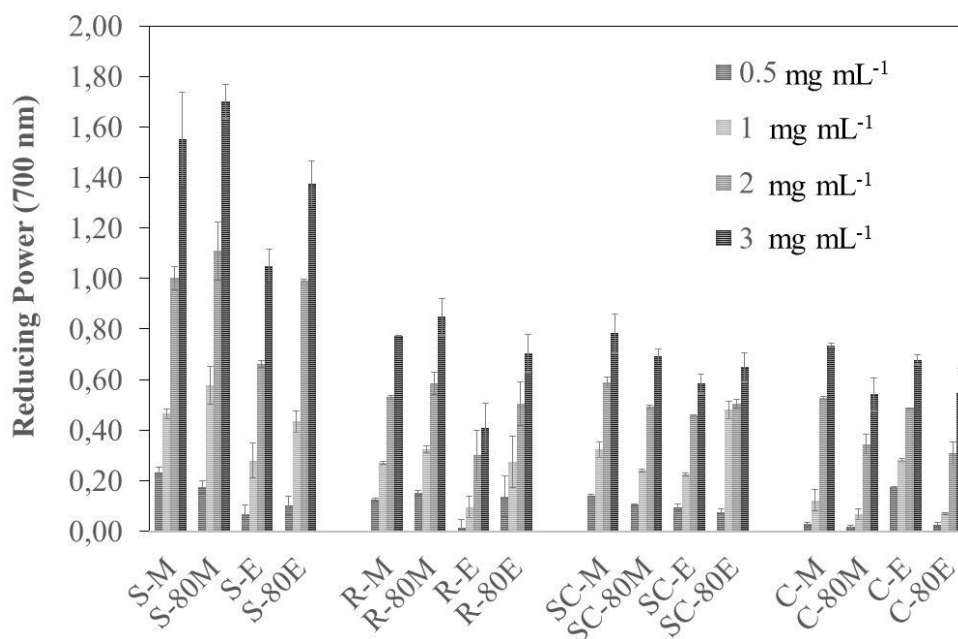
De Souza et al. (2014) divided the phenolic content into three separate groups: low content (<100 mg GAE 100 g⁻¹ fw), medium (100-500 mg GAE 100 g⁻¹ fw) and high (> 500 mg GAE 100 g⁻¹ fw). According to this statement, cornelian cherry had lowest phenolic content among all studied fruits. Otherwise, all extracts in this study had generally medium phenolic content. Besides, strawberry had closer values to the upper limit of medium phenolic content. Sorting to higher to lower were as follows: strawberry (361.00 mg 100 g⁻¹ fw) > sour cherry (199.54 mg 100 g⁻¹ fw) > raspberry (167.33 mg GAE 100 g⁻¹ fw) > cornelian cherry (114.14 mg 100 g⁻¹ fw).

Ferric Reducing Antioxidant Power

The method based on the electron transfer reaction between Fe³⁺ and Fe²⁺ in a ferric salt, which is an oxidant agent by the antioxidant compound. A higher absorbance shows a higher reducing power (Piccolella et al., 2008).

Reducing power of all extracts were shown in Figure 1. In all samples, reducing power increased with concentration (P<0.05), and this increase was almost doubled between 1 and 2 mg mL⁻¹

concentration. The effects of extraction solvent on the reducing power of the extracts was found significant ($P < 0.05$) at the studied concentrations. Overall, among all extracts, strawberries had the highest reducing power and have almost more than twice the others. Also, the reducing power of strawberry ethanol extracts (S-E and S-80E) was lower than that of methanol (S-M and S-80M) and the strawberry extract prepared with absolute ethanol (S-E) had the lowest reducing power among strawberry extracts. Reducing powers of strawberry extracts were higher than 1.00 absorbance at 3 mg mL⁻¹ concentration and ranged from 1.050 to 1.701 absorbance.



Analyses were done in triplicate. S-M, strawberry methanol; S-80M, strawberry 80% methanol; S-E, strawberry ethanol; S-80E, strawberry 80% ethanol; R-M, raspberry methanol; R-80M, raspberry 80% methanol; R-E, raspberry ethanol; R-80E, raspberry 80% ethanol; SC-M, sour cherry methanol; SC-80M, sour cherry 80% methanol; SC-E, sour cherry ethanol; SC-80E, sour cherry 80% ethanol; C-M, cranberry methanol; C-80M, cranberry 80% methanol; C-E, cranberry ethanol; C-80E, cranberry 80% ethanol.

Figure 1. Reducing power of extracts.

R-E had the lowest reducing power compared to all fruit extracts at all analyzed concentrations. The highest reducing power values among raspberry extracts were belonged to R-80M and the values were between 0.151 and 0.848.

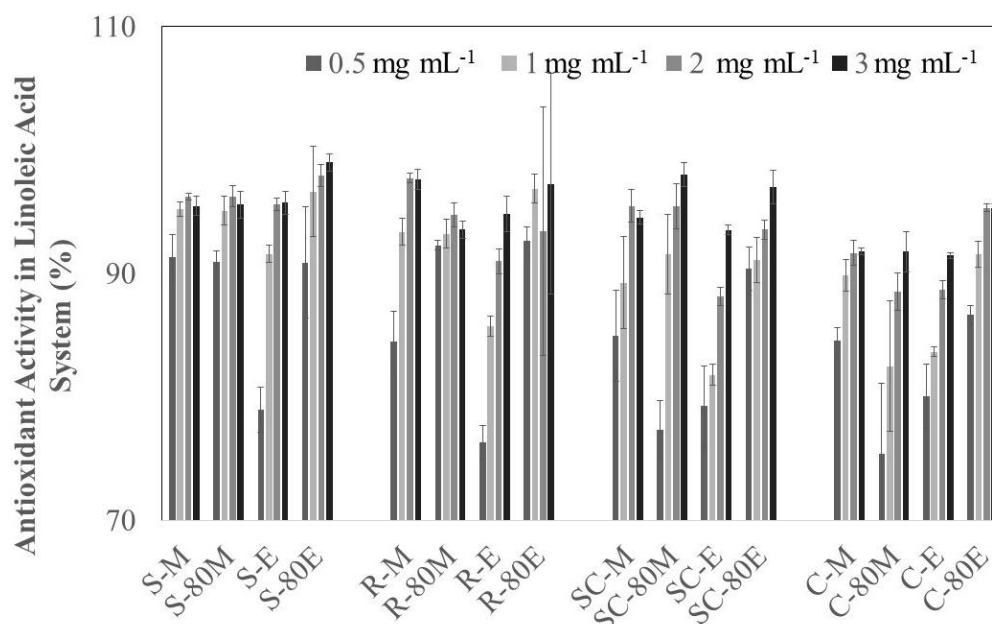
Reducing power of sour cherry extracts was the highest in SC-M (between 0.139 and 0.783) and the lowest in SC-E (between 0.095 and 0.584). Sour cherry had higher reducing power than cornelian cherry for the same solvent. Reducing power of C-80M and C-80E were quite low at 0.5 and 1 mg mL⁻¹ compared to all fruit extracts at that concentration. Piccolella et al. (2008) determined reducing power of sour cherry methanol, ethyl acetate, and hexane extracts at different concentrations (12.5-500 µg mL⁻¹) and it was calculated as % of the control sample. Methanol, ethyl acetate, and hexane extracts had reducing power between 6.8 and 79.8, 20.3 and 80.7, 4.9 and 39.9, respectively. Reducing power of methanol extract was lower than ethyl acetate extracts at 12.5 and 25 µg mL⁻¹ and the values got closer to each other as the concentration increased. While an increase in concentration caused slightly higher antioxidant activity in the more polar solvents (methanol and ethyl acetate extracts), it was not the same for hexane extract.

However, at 0.2 mg mL⁻¹ concentration, reducing power of BHA, BHT, and α -tocopherol were 2.095±0.015, 1.288±0.171, and 0.819±0.034, respectively. However, reducing power of extracts was lower than that of the BHA and BHT in the concentration range tested except strawberry extracts.

Strawberry extracts over 2 mg mL^{-1} concentration had higher reducing power values than α -tocopherol and at 3 mg mL^{-1} concentration they had close reducing power to that of BHT in this study. Also, R-80M had slightly higher reducing power than α -tocopherol.

Antioxidant activity in the linoleic acid system

An overall increase in antioxidant activity was observed with increased concentration ($P < 0.05$, Figure 2) except R-80E extract. The effects of solvent type on the antioxidant activities of the extracts were found significant ($P < 0.05$) for all concentrations except red raspberry extracts at 3 mg g^{-1} concentration. At the highest concentration, the values of all fruit extracts were similar except cornelian cherry extracts. Antioxidant activity of aqueous ethanol extracts was generally higher than those of the methanol extracts. Among all fruit extracts, S-80M had the highest antioxidant activity at 3 mg mL^{-1} . Other extracts with the highest antioxidant activity were R-M at 2 and 3 mg mL^{-1} , R-80E at 3 mg mL^{-1} , SC-80M and SC-80E at 3 mg mL^{-1} and the values were close to that of BHT ($98.65 \pm 1.12\%$). Additionally, aqueous ethanol extracts had higher antioxidant activity in linoleic acid emulsion among fruit extracts at all concentrations.



Analyses were done in triplicate. S-M, strawberry methanol; S-80M, strawberry 80% methanol; S-E, strawberry ethanol; S-80E, strawberry 80% ethanol; R-M, raspberry methanol; R-80M, raspberry 80% methanol; R-E, raspberry ethanol; R-80E, raspberry 80% ethanol; SC-M, sour cherry methanol; SC-80M, sour cherry 80% methanol; SC-E, sour cherry ethanol; SC-80E, sour cherry 80% ethanol; C-M, cranberry methanol; C-80M, cranberry 80% methanol; C-E, cranberry ethanol; C-80E, cranberry 80% ethanol.

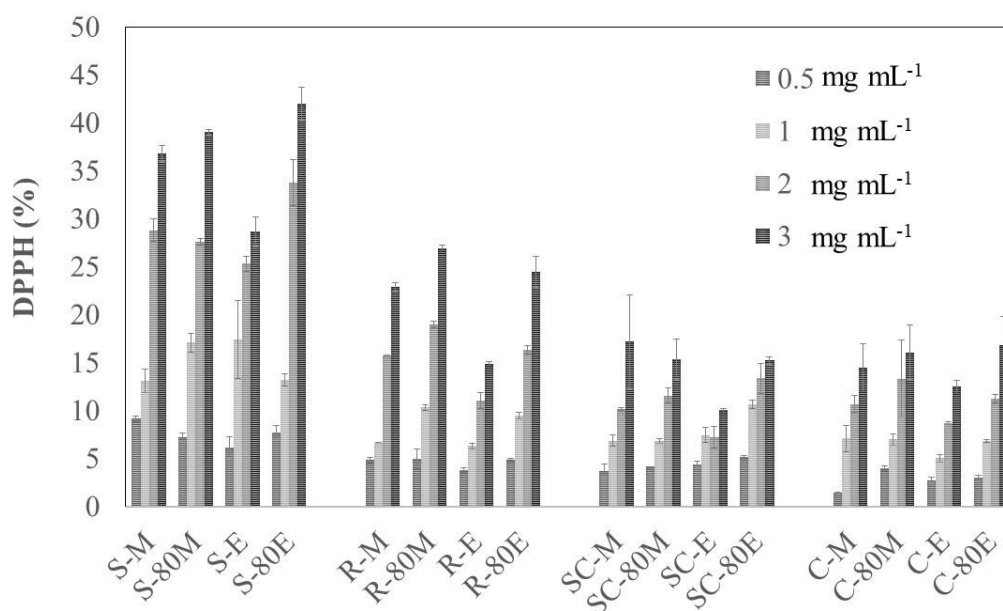
Figure 2. Antioxidant activity of extracts in linoleic acid system (%)

All strawberry extracts showed high antioxidant activities in the linoleic acid model system (78.9-99.0%). The antioxidant activity of all strawberry extracts above 1 mg mL^{-1} concentrations was very close to each other and above that of BHA and BHT (95.70 ± 1.72 and 98.65 ± 1.12 , respectively). While the antioxidant activity of R-M and R-E extracts were lower at lower concentrations (0.5 and 1 mg mL^{-1}) compared to R-80M and R-80E, the values were close to each other as the concentration increased. Antioxidant activity of aqueous ethanol extracts was 97.3, 97.0, and 95.3% for raspberry, sour cherry, and cornelian cherry extracts at 3 mg mL^{-1} , respectively. At the same concentration, C-80E had the highest value among cornelian cherry extracts. In addition, C-80M has the lowest values among all fruit extracts at 0.5 and 1 mg mL^{-1} .

DPPH Radical Scavenging Activity

The method is based on measuring the ability to reduce the DPPH radical, a stable compound in purple. When the purple-colored DPPH[•] radical solution is mixed with the extract with antioxidant activity, the antioxidant compound gives a hydrogen atom to the environment, forming a stable form of DPPH. So, intense purple color (DPPH[•]) disappears simultaneously and yellow color (DPPH) occurs as a result of reduction (Celep et al., 2012; Chaves et al., 2017).

As with other tests, concentration-dependent change was observed ($P < 0.05$, Figure 3). Scavenging effects of strawberry extracts on DPPH radicals were ranged from 6.11% to 41.97% in the concentration range tested. Strawberry extracts have the highest values for the same concentration among all extracts followed by raspberry extracts. R-80M and R-80E had higher values than R-M and R-E, although they have similar values in the beginning. Except for strawberry extracts, raspberry aqueous methanol extract (5-29%) had the highest DPPH radical scavenging activity at all the concentration tested, followed by C-80M (4.2-15.4%) and SC-80M extracts (4-16%).



Analyses were done in triplicate. S-M, strawberry methanol; S-80M, strawberry 80% methanol; S-E, strawberry ethanol; S-80E, strawberry 80% ethanol; R-M, raspberry methanol; R-80M, raspberry 80% methanol; R-E, raspberry ethanol; R-80E, raspberry 80% ethanol; SC-M, sour cherry methanol; SC-80M, sour cherry 80% methanol; SC-E, sour cherry ethanol; SC-80E, sour cherry 80% ethanol; C-M, cranberry methanol; C-80M, cranberry 80% methanol; C-E, cranberry ethanol; C-80E, cranberry 80% ethanol.

Figure 3. DPPH radical scavenging activity (%) of extracts

DPPH radical scavenging activity of SC-80E was higher than other extracts of sour cherry except at 3 mg mL⁻¹ concentration and the highest value at 3 mg mL⁻¹ concentration were belong to SC-M in this study. Piccolella et al. (2008) determined that DPPH scavenging activity of sour cherry methanol and ethyl acetate extracts at the lowest concentration were 32.6 and 38.4%, respectively. While the concentration of the ethyl acetate extracts increased, DPPH scavenging activity was increased rapidly, but there was a softer increase in the methanol extract. They stated that saccharidic components in methanol extracts may have an inhibitory effect on antioxidant activity.

In addition, while sour cherry extracts at 0.5 mg mL⁻¹ (between 3.69 and 5.19) had higher values than cornelian cherry extracts (between 1.39 and 3.98), the values were closer to cornelian cherry extracts as the concentration increased. Hassanpour et al. (2011) stated that methanol extracts of six different cornelian cherry had DPPH scavenging activity between 38.98 and 82.37%.

DPPH radical scavenging activity of BHA, BHT and α -tocopherol were $49.96 \pm 1.75\%$, $12.81 \pm 2.52\%$ and $33.20 \pm 0.00\%$ at 0.2 mg mL^{-1} . In comparison to synthetic antioxidants, the values of the extracts at 3 mg mL^{-1} concentration were higher than that of the BHT but lower than that of the BHA.

Also, EC_{50} values were calculated as the concentration of extract that cause 50% decrease in DPPH radical concentration via Microsoft Excel Software from the curve of concentration-inhibition graph (Table.2).

In this study, EC_{50} values for strawberries were lower than other extracts. In other words, the concentration required for 50% inhibition was less in strawberry extracts than in other extracts. This suggested that strawberry extracts had better radical scavenging activity. It was reported that EC_{50} values of strawberry were 0.79 mg mL^{-1} for water extract (Mendes et al., 2011) and 0.81 mg mL^{-1} for 80% methanol extract (Huang et al., 2012). R-E (13.31 mg mL^{-1}) has the highest value among raspberry extracts. It was found that EC_{50} values of 15 different raspberry extracts ranged from 7.16 to 13.31 mg mL^{-1} (Chen et al., 2013a) and the values overlap with the data in this study. EC_{50} value of SC-E extract (28.11 mg mL^{-1}) has the highest value among all extracts. EC_{50} values of cornelian cherry were $725 \text{ } \mu\text{g mL}^{-1}$ for 80% methanol extract (Celep et al., 2012) and were between 0.29 and 0.69 mg mL^{-1} for acetone/methanol/water/formic acid (40:40:20:0.1) extracts of 24 species (Tural and Koca, 2008).

Table 2. EC_{50} values (mg mL^{-1}) of extracts by DPPH radical scavenging activity

Solvent	Strawberry	Red Raspberry	Sour Cherry	Cornelian cherry
Methanol	5.35	8.43	11.78	12.21
80% Methanol	5.08	7.16	13.07	11.90
Ethanol	6.55	13.31	28.11	15.51
80% Ethanol	4.59	8.43	14.41	11.30

CONCLUSION

In this study, a positive correlation was obtained between the total phenolic content and antioxidant activities of the extracts. In addition, antioxidant activities increased due to the increase in concentration of extracts. According to antioxidant activity and total phenolic content analysis, strawberry extracts showed higher antioxidant properties compared to other fruits investigated.

All fruits examined in this study had a different profiles and unique antioxidant capacity depending on these phenolic compounds. Other solvent systems may be examined to obtain phenolic extracts and phenolic profile or antioxidant activities may be compared in subsequent studies. Antioxidant effects can be examined individually by purifying phenolic compounds with different methods. Because the non-phenolic compounds (such as ascorbic acid, glucose, fructose) in the composition of the fruits may affect the determination of the phenolic content and antioxidant activity (Piccolella et al., 2008; Chaves et al., 2017).

Although there are many studies on the phenolic profile and antioxidant properties of these fruits used in this study, limited researches were in the literature about using these fruits in different food systems as an alternative for synthetic antioxidants. Because the use of synthetic antioxidants is not particularly preferred by consumers, recently. Additionally, antioxidant activities and their bioavailability of phenolic compounds in these fruits can be investigated in different model systems such as bulk oils or emulsions or their antioxidative effects in high-temperature applications against oxidation reactions such as rancimat analysis, frying, thermal storage analysis.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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