RESEARCH on THE PHENOLIC COMPOUNDS in SARILØP (FICUS CARICA L.) FIG VARIETY

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Abstract
Phenolic compounds are food components that have the features which are called anticancerogenic, antioxidative, antimutagenic, holding the free radicals and the inhibition of lipid peroxidation. Fig is an edible fruit that grows in the tropic and subtropic areas. Sarílop is a variety of fig that has long been associated with horticulture in the Egean region of Turkey. Ten samples of fresh fig and ten samples of dried fig supplied from different manufacturers in Turkey were analyzed to determine their total phenolic content, total flavonoid content, DPPH and FRAP radical scavenging activity, qualitative and quantitative phenolic compounds by HPLC. Gallic acid, chlorogenic acid, (-)-epicatechin, syringic acid, rutin and psoralen were determined in fresh and dried figs by HPLC. It was determined that the major phenolic compound is (-)-epicatechin. Statistically difference between different sorts of fig are significant (P < 0.05). The amount of polyphenol is higher in fresh figs compared to dried ones.

Keywords: Antioxidant activity, Ficus carica L., high pressure liquid chromatography (HPLC), phenolic compounds, Sarílop, total phenolic content.

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SARILOP (FICUS CARICA L.) İNCİR ÇEŞİDİİNİN FENOLİK BİLEŞİKLERİNİN ARAŞTIRILMASI

Özet
Fenolik bileşikler, antikanserojen, antioksidatif, antimutajenik, serbest radikalleri bağlama ve lipid peroksidsasyonunu önleme özelliklerine sahip gıda bileşenleridir. Incir, tropik ve subtropik bölgelerde yetiştirilen yenilebilir bir meyvedir. Sarílop ise, Ege bölgesinde yaygın olarak yetiştirilişi yapılan bir incir çeşididir. Türkiye’deki farklı üreticilerden temin edilen çeşitli yaş ve on çeşit kuru incir, toplam fenolik madde miktarlarını, toplam flavonoid içeriklerini, DPPH ve FRAP yöntemleriyle radikal süpürtücü aktivitelerini, HPLC cihazıyla kalitativ ve kuantitatif olarak fenolik bileşiklerini belirlemek amacıyla analiz edilmiştir. HPLC analizi sonucunda yaş ve kuru incirlerde galaktik asit, klorogenik asit, (-)-epiKatechin, siringik asit, rutin ve psoralen tespit edilmiştir. Incirdeki major fenolik bileşiğin (-)-epiKatechin olduğu saptanmıştır. Incir çeşitleri arasında istatistiksel açıdan analımlı bir farklılık vardır (P < 0.05). Yaş incirlerin fenolik içerikleri, kuru incirleri göre daha yüksektir.

Anahtar kelimeler: Antioksidan aktivite, fenolik bileşikler, Ficus carica L., Sarílop, toplam fenolik madde miktarı, yüksek performanslı svi kromatografisi (HPLC).
INTRODUCTION

The fig tree (*Ficus carica* L.) is cultivated for its fruit in warm and dry climates and the dried fruit has been a food familiar to human beings since B.C. 3000 (1).

Commercial production of fig is carried out in countries where the Mediterranean climate prevails, such as California, Australia and South America as well as Mediterranean countries. Turkey leads the fig production in the world. 65% of the fig trees are in the Western Aegean Region, especially in Small and Big Meander basins (2). From these basins, 75% of the fresh fig production and the total export goods of dried fig are supplied. The highest quality figs are grown in these basins because of the convenience of the region's conditions (3).

Figs are rich in calcium, potassium, ascorbic acid, vitamin A, dietary fiber, some fatty acids and many phenolic compounds (1). The basic polyphenols in figs are flavones (apigenin, astragalin, camphorol and rutin), catechin (catechin and epicatechin), flavonones, anthocyanidin (cyanidin), chlorogenic acid, gallic acid and syringic acid (4). It has been indicated that fig also contains psoralen (5).

Dietary fruits and vegetables provide a reasonable amount of compounds that act as physiological antioxidants. Antioxidant nutrients have important roles in preventing pathogenic processes related to cancer, cardiovascular disease, macular degeneration, cataracts, and asthma, and may enhance immune function (6). Antioxidant defenses protect the body from the detrimental effects of free radicals generated as by-products of normal metabolism (7).

Sarilop is a type of fig whose peels are thin and greenish yellow. The fruit weight is about 65-70 g. It is a type which can be harvested from late of July to late of September. It is an advantage for the point of drying technology and quality parameters, as the color of the dried fruit is whitish yellow, the moisture ratio is 22-24% and the sugar ratio is 50-55% (8). It is widely grown in the Aegean Region, particularly in Aydın and Izmir. It is a type officially registered by Aydın Chamber of Commerce. 90% of the Turkey’s fig production is composed of Sarilop type fig. 15-20% of the figs produced are exported (9).

In this study, the phenolic compounds of fresh and dried figs grown in 10 different districts (in Aydın and Izmir) have been analyzed with spectroscopic and chromatographic methods. The aim of the present study is to figure out the effect of the region grown on the phenolic content of fresh and dried Sarilop type figs.

MATERIALS and METHODS

Materials

Samples

Ten types of figs are Sarilop fresh figs and other ten types of them are Sarilop dried figs, totally twenty types of fig were analyzed. Five types of dried and fresh figs were supplied from Erbeyli Fig Research Institute. The samples supplied from Erbeyli Fig Research Institute were from the cultivar Incirliova type 1-5. The rest figs were supplied from various districts of Aydın (Germencik, Çine, Nazilli, Söke) and from Ödemiş that’s been located in İzmir.

Methods

Determination of Total Moisture Content

All of the fresh figs were homogenized by using Waring commercial blender and by using meat mincer for dried figs before all analyses. Total moisture content of fresh and dried figs was determined by using vacuum-operated oven. The samples were dried at 65°C to a constant weight (10).

Extraction of Phenolic Compounds

The samples were extracted in terms of the method described by García-Salas et al. (2010) (11) and used for spectrophotometric analyses. The extract of phenolic compound was obtained according to Veberic et al. (2008) (12) method and used for determining phenolic compounds in the figs by high pressure liquid chromatography analysis.

Determination of Total Phenolic Content

The total phenolic content of the extracts was determined by a Folin-Ciocalteu phenol reagent method (13) using gallic acid as standard. 50 µl of the extracts, 3 ml of distilled water, 250 µl of Folin-Ciocalteu reagent and 750 µl of 7% Na₂CO₃ were mixed and vortexed for 30 sec. Then, the mixture was incubated for 8 min at room temperature and the distilled water (950 µl) was added. After the mixture was stood for 2 hours at room temperature and dark, the absorbance
was measured with UV/vis spectrophotometer (Varian Cary 50 Scan, Australia) at 760 nm. A mixture of 80% methanol and reagents was used as a blank solution. The total phenolic content was expressed as gallic acid equivalents (mg of GAE/100 g dry matter) through the calibration curve of gallic acid that its linearity range was 50-700 µg/ml ($R^2$>0.99).

**Determination of Total Flavonoid Content**

The total flavonoid content was determined using Heimler et al. (2005) (14) method. 250 µl of extract or rutin standard solution or 80% metanol (blank solution) was mixed with 1.25 ml of distilled water and 75 µl of 5% NaNO$_3$ solution. The mixture was vortexed for 15 sec and stood for 6 min at room temperature. 10% AlCl$_3$.6H$_2$O (150 µl) was added on the mixture and then, it was incubated for 5 min at room temperature. 0.5 ml of 0.1 M NaOH solution and 275 µl of distilled water were added and the mixture was vortexed for 20 sec. The absorbance was measured at 510 nm immediately. Total flavonoid content was expressed as rutin equivalents (mg of RE/100 g dry matter) through the calibration curve of rutin that its linearity range was 25-350 µg/ml ($R^2$>0.99).

**Determination of Antioxidant Activity by The DPPH Radical Scavenging Method**

DPPH radical scavenging capacity of fig extracts was performed in terms of the methods of Sun et al. (2007) (15) and Cemeroğlu (2007) (16) with some modifications. 15, 30, 45 µl of sample and 30 µl of Trolox were completed to 2 ml with 0.1 mM DPPH. The mixture was vortexed for 20 sec. The absorbance was measured at 515 nm after 20 min incubation at room temperature and dark area. 2 ml of 80% methanol was used as a blank solution. The absorbance of DPPH (2 ml) was $A_{control}$. The inhibition percentage of the absorbance was calculated as follows: Inhibition % = ($A_{control} - A_{sample}$)/$A_{control}$. The antioxidant activity was expressed as Trolox equivalent (mg Trolox/100 g dry matter). It was the ratio between the slope of the inhibition % versus amount of sample and that of Trolox. Linearity range of the calibration curve of Trolox was 0.1 to 1.0 mM ($R^2$>0.99).

**Determination of Antioxidant Activity by The FRAP Radical Scavenging Method**

The FRAP assay was evaluated as described by Guo et al. (2003) (17) and Xu et al. (2004) (18) with slightly modifications. 2.5 ml of 10 mmol/L TPTZ solution in 40 mmol/L HCl, 2.5 ml of 20 mmol/L FeCl$_3$ solution and 25 ml of 0.3 mol/L pH 3.6 acetate buffers were mixed. The mixture was prepared, then warmed to 37°C and described as the working FRAP reagent. 40 µl of fig extract or standard (FeSO$_4$ solution) was added to 1.8 ml FRAP solution and 200 µl distilled water. The mixture was stood for 30 min at 37°C. The absorbance was read at 593 nm. The FRAP value of samples was calculated as FeSO$_4$ equivalents (mg FeSO$_4$/100 g dry matter). Linearity range of the calibration curve of FeSO$_4$ was 0.2 to 3.0 mmol/L ($R^2$>0.99).

**Qualitative and Quantitative Determination of Individual Phenolic Compounds in Fig Extracts by HPLC**

The polyphenols were analyzed in HPLC according to the method described by Çam (2009) (19) with some modifications. The extracts of fresh and dried figs were analyzed on Agilent 1200 (USA) HPLC system with a diode array detector. Hichrom C$_{18}$ (4.6 mmx250 mm; 5 µm particle size) column, 40°C column temperature and 20 µl injection volume were used in the analysis. The solvent system was a gradient of water-acetic acid (98:2) (A) and methanol (B). The gradient employed was: starting with 95% A, from 95% A to 50% for 10 min, from 50% A to 30% for 5 min at a flow rate of 1.0 ml/min. The phenolic compounds were monitored at 272 nm for gallic acid, 275 nm for (-)-epicatechin, 279 nm for chlorogenic acid, syringic acid, psoralen and 356 nm for rutin. The spectra of these polyphenols were recorded between 190-400 nm. Gallic acid, (-)-epicatechin, chlorogenic acid, syringic acid, psoralen and rutin (Fig. 1) were determined qualitatively by comparing retention times and spectra of standards and quantitatively by using external standards method. The HPLC method was validated. Due as et al. (2008) (20) reported that the limit of detection (LOD) and the limit of quantification (LOQ) are defined as 3:1 and 10:1 peak to noise ratio. LOD and LOQ were calculated for gallic acid, chlorogenic acid, syringic acid, rutin, (-)-epicatechin and psoralen.

**Statistical Analysis**

All the experiments, except total moisture content analysis, were performed in duplicate.
Total moisture content analysis was carried out triplicate. Statistical analyses were realized with the SPSS 15.0 statistics package program. The statistical analyses of the data were achieved by using one-way analysis of variance (ANOVA) and Duncan post-test. In all data analyses a value of P< 0.05 was considered as statistically significant.

RESULTS and DISCUSSION

Total Phenolic Content

In the performed study, the amount of the total phenolic compounds was expressed as gallic acid equivalent in Table 1-a and Table 1-b. Among the fresh figs, while İncirliova type-2, İncirliova type-4, Germencik cultivar figs had the highest amount of total phenolic compound, İncirliova type-1, İncirliova type-3, İncirliova type-5, Çine, Nazilli, Ödemiş, Söke cultivar figs had the lowest phenolic content. Among dried figs, the highest amount of phenol content was in İncirliova type-3, İncirliova type-5 cultivar figs and the lowest polyphenol content was in Germencik, Çine, Nazilli, Ödemiş cultivar figs.

In a study on the phenolic content of figs, it was determined that the total polyphenols content of dried figs grown in Turkey was 1.234±41 mg GAE/100 g DM (dry matter), and the total phenolic content of fresh figs grown in Japan was 1.699±24 mg GAE/100 g DM (21). As a result of the analysis of the figs harvested in Slovenia, it was determined that the highest amount of total phenolic content was in "Miljska figa" cultivar fig (average 41.87±1.17 mg GAE/100 g) and the lowest amount was in "Bela petrovka" cultivar fig (average 18.41±1.04 mg GAE/100 g) (22).

Total Flavonoid Content

In this study, the amount of total flavonoids was expressed as the rutin equivalent (Table 1-a and Table 1-b). According to the results of the analysis of total flavonoid amount, among the fresh figs, İncirliova type-1, İncirliova type-3, Germencik cultivar figs had the highest and İncirliova type-5, Çine cultivar figs had the lowest amount. Among dried figs, the highest amount of flavonoid content was in İncirliova type-1, İncirliova type-3, İncirliova type-5, Söke cultivar figs and the lowest flavonoid content was in İncirliova type-2, İncirliova type-4, Germencik, Çine, Nazilli, Ödemiş cultivar figs.

In literature, there are only two studies determining the total flavonoid amount in the fig. The first of these was provided by Yang et al. (2009) (23). By two different extraction methods they used in
their study, the analysis results of the total flavonoid amount of fresh figs were respectively determined as 184 mg CE (catechin equivalent)/100 g and 167 mg CE/100 g. In the second study, Solomon et al. (2006) (24) determined the total flavonoid amount between 21.5 mg CE/100 g FW (fresh weight) (Mission) and 2.1 mg CE/100 g FW (Kadota). In this study, the results belonging to the total phenolic compounds and total flavonoids obtained from figs were quite high compared to the literature data. The extraction method used, analysis parameters, the place where the samples were supplied, the fig type, the term of the expressed results closely have affected the results of the analysis whether the results were expressed on dry matter or not has also affected the results. The result of the analysis expressed that the total phenolic content amount of Sarıloq type of fresh and dried fig grown in Aydın was higher than many of the other figs grown in different countries. Sarıloq type figs, which were rich in total phenolic content amount, were expected to be rich in flavonoid compounds. There was no comparison because there weren’t any studies determining the total flavonoid amount of dried figs in literature.

Antioxidant Activity by The DPPH Radical Scavenging Method

Using DPPH method, the result of the analysis of antioxidant activity was determined as trolox equivalent antioxidant capacity (TEAC) (Table 1-a and Table 1-b). According to the data received, it was determined that the Incirliova type-1 cultivar fresh fig had the highest and Incirliova type-4 and Nazilli-origin figs had the lowest antioxidant activity. The highest antioxidant activity values among dried figs were in Incirliova type-3 cultivar fig and the lowest antioxidant activity values were in Germencik, Çine and Nazilli-origin figs.

In the studies of Serteser et al. (2009) (25), it was found that the antioxidant activity value of fresh fig was 1.562 mg sample/mg DPPH in "IC$_{50}$". In another study, it had been determined that the antioxidant activity of dried fig grown in Turkey was 1.087±11 mg AEAC/100 g DM (ascorbic acid equivalent antioxidant capacity in per 100 g dry matter), the antioxidant activity of fresh fig grown in Japan was 2.524±37 mg AEAC/100 g DM (26).

In literature, the DPPH method used the results of antioxidant activity expressed in terms of "IC$_{50}$" or "ascorbic acid equivalent antioxidant capacity (AEAC)". So the results were not compared with the literature.

Antioxidant Activity by The FRAP Radical Scavenging Method

The analysis results of the antioxidant activity performed with FRAP method were calculated in terms of FeSO$_4$ equivalent in Table 1-a and Table 1-b. When the analysis results were evaluated, it was observed that the Incirliova type-1, Incirliova type-4, Germencik and Nazilli cultivar figs had the highest antioxidant activity and Çine cultivar fig had the lowest. In dried figs, the highest antioxidant activity was in Incirliova type-2, Incirliova type-3, Incirliova type-5 cultivar figs and the lowest was in Incirliova type-1, Incirliova type-4, Germencik, Çine, Nazilli, Ödemiş, Söke cultivar figs.

In their studies, Çalışkan and Polat (2011) (27) had found the value of antioxidant activity as 5.9±1.2 mmol Fe$^{2+}$ equivalent/kg FW (89.67 mg FeSO$_4$/100 g) in the yellow peel fig they supplied from Hatay. The antioxidant activity values found in literature were quite low comparing to the values obtained from this study. This difference might be the results of researches in the literature were not expressed in terms of dry matter. But even the result was given in terms of dry matter, this difference would not disappear. In this case, it was understood that the antioxidant activity of the fresh figs grown in Aydın was higher than the figs grown in Hatay. Because there wasn’t any current study in the literature that the antioxidant activity value of the dried figs were obtained from FRAP method, the results of this study could not be compared to the values taken from dried figs.

High Pressure Liquid Chromatography (HPLC) Analysis for Phenolic Compounds

Eleven different concentrations were used for calibration curves in high pressure liquid chromatography analysis. The working concentration range of standards was from 0.834 to 83.34 mg/L approximately. Correlation coefficients were greater than 0.99 for all phenolic compound standards. The limit of detection and the limit of quantification were calculated for gallic acid, chlorogenic acid, syringic acid, (-)-epicatechin,
rutin and psoralen. The analytical recovery was determined by the original amount of phenolic compound in fresh and dried figs with the standard phenolic compound added. The original amount of phenolic compound in the figs was added before extraction of phenolic compounds into Ödemiş cultivar fresh and dried figs. The recovery factors and the values of LOD and LOQ are showed in Table 2.

The results of chromatographic analysis were expressed in "mg/100 g DM" terms. It was observed that high amount of phenolic compound in fresh figs was (-)-epicatechin and followed up with rutin in Table 3-a. It was determined that except from Incirliova type-5, Çine and Ödemiş cultivar figs, in all fresh figs, phenolic compound which had the lowest amount was gallic acid. In Incirliova type-5 cultivar figs psoralen, in Çine cultivar figs chlorogenic acid, in Ödemiş cultivar figs, syringic acid had the lowest content.

The highest amount of phenolic compound found in dried figs was (-)-epicatechin. It was followed by rutin in Table 3-b. It was determined the lowest amount of phenolic compound found in figs other than Incirliova type-3 and Söke cultivar figs was syringic acid. The lowest amount of phenolic compound was chlorogenic acid in Incirliova type-3 cultivar figs and gallic acid in Söke cultivar figs.

In literature, we came across quite few studies in which the phenolic compounds of the fig were determined qualitatively and quantitatively. In the study carried out by Veberic et al. (2008) (12) as a result of the analysis made by high-pressure liquid chromatography of the fresh figs harvested in Slovenia, the gallic acid content was found between 0.15-0.38 mg/100 g FW, the chlorogenic acid content was found between 0.46 – 1.71 mg/100 g FW, the (-)-epicatechin content was found between 0.34 – 0.97 mg/100 g FW, the syringic acid content was found between 0.22 – 0.104 mg/100 g FW and the rutin content was found between 4.89 – 28.7 mg/100 g FW.

Another study was performed in Portugal. As a result of a study based on two types of figs grown in Portugal, the chlorogenic acid content of "Pingo de Mel" type fig peels was 3.2±0.9 mg/kg lyophilized extract (0.32±0.09 mg/100 g), pulp rutin content was 499.1±1.2 mg/kg lyophilized extract (49.91 mg/100 g) and psoralen content was 6.3±0.5 mg/kg lyophilized extract (0.63 mg/100 g). In "Branca Tradicional" type figs, it was determined that rutin content was 30.8±1.2 mg/kg lyophilized extract (3.08 mg/100 g) and psoralen content was 35.4±2.8 mg kg (3.54 mg/100 g) lyophilized extract (5).

In this study, it was obvious that the phenolic compound levels of Sanlo type figs were higher than the literature. Only the rutin content of Sanlo fig was compatible with the literature. Because there weren’t any studies in literature determining the phenolic compound of dried fig, the obtained results could not be compared with the values in literature.

If it was needed to evaluate on the basis of regions, total phenolic content and total flavonoid amounts of all dried figs were lower than the fresh figs; because the ratio of aglycon was lower in dried figs. It was emphasized in the literature that during the drying process, comparing to glycosides, aglycons were more impaired and less stable (26). As it was expected, in figs belonging to all regions, total phenolic content amount was higher than the total flavonoid amount; because in total flavonoid content analysis, the amount of phenolic acids in fruit were also calculated besides flavonoids. Antioxidant activity values of all figs obtained by FRAP method were lower than the antioxidant values of values obtained by DPPH method. In literature there were similar results. The difference between the results was due to the FRAP method that could not measure glutathione which has a thiol group, an important antioxidant in live plants and animal cell; and because of different standards used in each FRAP and DPPH methods. Except from

Table 2. LOD, LOQ and recovery values in high pressure liquid chromatographic analysis

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>LOD [mg/L]</th>
<th>LOQ [mg/L]</th>
<th>Recovery for Ödemiş cultivar fresh fig [%]</th>
<th>Recovery for Ödemiş cultivar dried fig [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.25</td>
<td>0.84</td>
<td>97.55</td>
<td>94.17</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.30</td>
<td>0.99</td>
<td>98.55</td>
<td>97.09</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>0.26</td>
<td>0.85</td>
<td>96.40</td>
<td>99.53</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.30</td>
<td>1.00</td>
<td>99.42</td>
<td>93.09</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.39</td>
<td>1.29</td>
<td>96.13</td>
<td>94.48</td>
</tr>
<tr>
<td>Psoralen</td>
<td>0.32</td>
<td>1.08</td>
<td>96.97</td>
<td>96.70</td>
</tr>
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</table>
Research on The Phenolic Compounds in...

In all fresh figs, the amount of all phenolic compounds (gallic acid, chlorogenic acid, (-)-epicatechin, syringic acid, rutin, psoralen) determined by HPLC were higher than dried figs. In literature, the reason of this was explained as the demolition of some polyphenols of the fresh figs during the drying process or the transformation to non antioxidant forms (28).

**CONCLUSIONS**

According to the results of this study, the phenolic content of the fresh figs grown in Aydın-Izmir region was relatively higher than the phenolic contents of the figs grown in world's other countries. Because of high-phenolic content of fig, it can be called as a functional food. This tag will bring about different approaches to fig. In addition, because of its higher phenolic compound content, Sarılop type figs, accordingly Aegean Region and especially Aydın-Izmir Region will be heard all around the world. With this study, the phenolic compounds of dried figs were brought to light for the first time and it will guide the next coming studies.

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