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DETERMINATION OF PHYTOCHEMICALS FROM FRESH FRUITS OF FIG (*Ficus carica* L.) AT DIFFERENT MATURITY STAGES

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ABSTRACT

The natural and ubiquitous production of figs throughout Turkey makes it one of the most important centers of fig genetic resources. The current study aims to determine the most important phytochemical ingredients of a local variety of fig (*Ficus carica* L.) collected from the natural habitats in the province of Kahramanmaras at different harvest intervals in 2018. The fruit samples were assayed for various metabolites such as phenolic compounds, total phenols (TP), total flavonoids (TF), total anthocyanins (TA) and total antioxidant capacity (TAC). The results showed that epicatechin (7.809 mg/100 g FW) was dominant phenolic compound in the fruits of this variety, followed by myricetin (2.632 mg/100 g FW), kaempferol (2.396 mg/100 g FW) and quercetin (0.655 mg/100 g FW). The average values obtained for TP, TF, TA, and TAC were found to be 135.71 mg GAE/100 g FW, 188.20 mg/100 g FW, 54.65 ml/L, and 14.34 DPPH%, respectively. There were also observed significant differences in total polyphenolic, flavonoid, anthocyanin content, and the antioxidant capacity at different harvest periods. The current findings indicate that the fig variety explored has a high antioxidant activity and is rich sources of anthocyanins and phenolic content. This study provides valuable information about the health benefits of figs endorsed by the phytochemical characteristics.

Key words: *Ficus carica* L., anthocyanins, antioxidant potential, phenols

INTRODUCTION

Fig (*Ficus carica* L) represents as one of the most important horticultural groups belonging to the family, Moraceae. Owing to its wide ecological adaptability and despite being a subtropical or tropical fruit, it can also thrive in a temperate climate. Figs are consumed fresh as well as dried in countries such as Turkey, Greece, USA, Italy, Portugal, and Spain. Turkey is the world leader in the production and export of both dried and fresh figs and possesses a rich genetic diversity. Among the major economically important fig varieties, Bursa Black and Aydin (Yellow lop) cultivars are the most popular. In addition to these varieties, there are also available many other local varieties of great climatic and economic importance. However, in today's era of trade and competition, adequate attention has not been paid toward uplifting the productivity and quality of fresh figs to meet the global demand. The province of Kahramanmaras in the Eastern Mediterranean Region, in particular, the Fatmali region, has an appropriate environment and soil configuration for 'Abbas' variety. The variety developed through intense cultivation in this geographical region has its unique taste and a rare-fig potential due to the special ecology. The variety constitutes a major part of the present fig production areas in Kahramanmaras. Abbas figs are preferred as a table fruit due to the desirable size and taste. In recent years, there has been noticed a rapid increase in efforts toward improving nutritional value, storage conditions,

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and transportation opportunities especially in countries where the fig does not grow as an exotic fruit. Therefore, both pomologists and consumers have been showing a growing interest due to their contribution to healthy living. Fruits and vegetables, a rich source of metabolites such as flavonoids and phenolics, exhibit various protective effects [Serce et al. 2010, Alibabic et al. 2018]. Most of these phytochemicals show antioxidant activity and can help in protecting the cells from oxidative stress caused by free radicals [Solomon et al. 2006, Veberic et al. 2008, Chang et al. 2016]. They are also a valuable source of naturally occurring antioxidants, of which phenolics and flavonoids play a vital role in preventing health disorders related to oxidative stress, including cardiovascular diseases [Eberhardt et al. 2000, Slatnar et al. 2011, Kamiloglu and Capanoglu 2015]. Phenolic compounds occur as various secondary metabolite groups in fruit, seeds, flowers, leaves, branches, and stems of plants. They have been proved to exhibit anti-aging effects attributed to the antioxidant properties by removing free radicals. In addition, antioxidants strongly affect the color and aroma of foods. In recent years, huge data have been generated on the presence of polyphenol compounds in a variety of food materials, including figs [Solomon et al. 2006, Kamiloglu and Capanoglu 2015]. The corresponding antioxidant activities are determined using different methods depending on the phenolic compounds, flavonoids, anthocyanins, and their extraction [Solomon et al. 2006, Duenas et al. 2008, Caliskan and Polat 2011]. Antioxidants are very important for the human body as they protect the cells against free radicals, which result in many oxidative processes. Antioxidants play an essential protective role against oxidative degradation. Anthocyanins and phenols are very expedient compounds for human health as they alleviate the harmful effect of free radicals [Duthie et al. 2000]. Besides, phenols also make up the color, taste, and aroma in fruits and vegetables [Veberic et al. 2008]. Solomon et al. [2006] showed that the higher polyphenol content, particularly anthocyanins, in fig fruit, leads to their higher antioxidant activity. The functional activity of these compounds is mainly expressed as their free radical scavenging potential, which is involved in certain pathological conditions [Briviba and Sies 1994]. There are available several reports describing the distribution of the phenols in fig pulp and peel [Solomon et al. 2006, Del Caro and Piga, 2008, Pourghayoumi et al. 2017]. This is the first attempt of assessing the phytochemical potential of 'Abbas' a local variety and determining the effect of harvest time on the bioactive profile. Despite the immense importance of polyphenolics and their antioxidant potential, unfortunately, there are no data available on antioxidant properties and phenolic compounds from Turkish figs so far. Therefore, the present study was conducted in order to determine the important physicochemical characteristics such as total phenolic content, total flavonoids, and antioxidant capacity and also the phenolic profiling of fruit pulp from 'Abbas' variety grown widely in the Kahramanmaras province of Turkey at different harvest intervals.

MATERIAL AND METHODS

Material

The city of Kahramanmaras is located between 37°43' north longitude and 37°8' east latitude and at an altitude of 900 m above the sea level. It has a continental climate with the highest average temperature in August (35.9°C) and the lowest average temperature in January (1.2°C). The local fig variety, developed during a selection project, was obtained as a research parcel from TAGEM (General Directorate of Agricultural Research and Policy). The fruits were collected at different maturity stages from the research and experimental area of Kahramanmaras provinces of the Mediterranean region of Turkey in the year 2018 (Figs 1 and 2). Probably, other closely related varieties also grow in the Mediterranean basin but possess different characteristics and represent local types of figs. The fruits were sampled as pulp (Fig. 3). Harvesting was done at 10-day intervals of commercial maturity stage in the period June-August 2018 (Tab. 1). About 10 kg of mature fresh fruits were randomly chosen and packed on the ice and immediately transported to Laboratory of Department of Horticulture, Faculty of Agriculture, University of Cukurova. For each analysis, fresh figs were randomly chosen in 10 replicates and the peel was manually separated from the pulp. About 10 grams of the homogenized samples were taken in falcon tubes and stored at –20°C until analyses. All samples were screened for their phenolic compounds, total phenolic content, total flavonoids, total anthocyanins, and total antioxidant capacity.

Fig. 1. General view of the research parcel (source: Turkish Land Registry and Cadastre Information System (TAKBiS) webpaige)

Fig. 2. General view of the research garden (photo: M.A. Gündeşli)

Table 1. Fig sampling dates for 'Abbas' variety

Local variety name	Harvest periods	Date (day/month/year)
ABBAS	first period	15.07.2018
	second period	25.07.2018
	third period	05.08.2018
	fourth period	15.08.2018

Determination of phenolic compounds

Phenolic compounds were determined by HPLC (High-Performance Liquid Chromatography) equipped UV and DAD detectors according to the method of Kosar et al. [2004]. The extraction of phenolic compounds was done by 10 mL of acetone/water (1 : 4, v/v) mixture containing 100 µL of trifluoroacetic acid. The separation was performed on a 5-µm reverse-phase Inertsil ODS–3 (C18) (4.6 mm, 250 mm) analytical column operating at 40°C at a flow rate of 1 mL/min.

Fig. 3. Images of 'Abbas' fig variety (photo: M.A. Gündeşli)

Detection wavelength were 280 nm and 360 nm. Elution was carried out using a nonlinear gradient of the solvent mixture 2.5% formic acid in water (solvent A) and 2.5% formic acid in acetonitrile (solvent B).

Determination of total phenolic content (TP)

Determination of total phenolic content of pulp was done by modifying the spectrophotometric Folin-Ciocalteu method developed by Spanos and Wrolstad [1990]. About 10 mL of methanolic extract of 1 g homogenized fig samples was used. The obtained values were expressed as milligrams of gallic acid equivalent in 100 g extract (mgGAE/100 g).

Determination of total flavonoid content (TF)

The total flavonoid contents were expressed as milligram rutin equivalents per gram of extract [Miliauskas et al. 2004]. Flavonoids were measured by mixing 1 mL of appropriate diluted methanolic extract with 1 mL of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 15 min, the absorbance was measured at 420 nm.

Determination of total antioxidant content (TAC)

The antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams et al. [1995], with some modifications [Duarte-Almeida et al. 2006]. A 50 µL aliquot extract and 250 µL of DPPH (0.5 mM) were mixed and the absorbance was measured at 517 nm using a Microplate Spectrophotometer after 20 min. The methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) at different concentrations served as the control. The

antioxidant capacity was expressed as μmoles Trolox equivalents per gram of sample in fresh weight (FW).

Determination of total anthocyanin content (TA)

The total anthocyanin content was determined according to the modified pH differential method [Cheng and Bren 1991]. Absorbance was measured at 510 and 700 nm, where absorbance of the sample was calculated as: $A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$ and expressed as milligram per liter (molar extinction coefficient of 29600 and molecular weight of 4455.2) per 100 g FW.

Statistical analysis

The experiments were designed as a completely randomized block including three trees for each replicate. Statistical analysis was performed using JMP statistical software from SAS (Version 7). Differences among the mean values were detected by the least significant differences (LSD) test at $p = 0.05$.

RESULTS AND DISCUSSION

Fig is an excellent source of minerals, vitamin, dietary fiber, and phenolic substances that contribute tremendously to a healthy life. Besides, other phenolic substances add significantly to the quality, taste, and aroma of fruits. Figs constitute an ideal supplementation to a human diet as an exceptional source of fibers and natural sweetness [Solomon et al. 2006, Veberic et al. 2005, 2008]. Previous reports have shown the importance of phenolics in food and especially fruits owing to their beneficial health effects [Trichopoulou et al. 2006, Herrera et al. 2009]. Significant differences were recorded between the phytochemical contents of the local fig variety 'Abbas' included in the study ($P < 0.05$) (Tabs 2 and 3). All results were expressed on a fresh weight basis. The pulp exhibited great diversity in the levels of different phenolic compounds, TP, TF, TAC, and TA (Tabs 2 and 3). The phenolics analyzed in our study were caffeic acid, catechin, myricetin, kaempferol, quercetin, ferulic acid, p-coumaric acid, gallic acid, benzoic acid, syringic acid, epicatechin, ellagic acid, naringenin and chlorogenic acid with a statistically significant level $p < 0.05$ (Tab. 3). The HPLC profile exhibited about 14 different phenolic compounds at different harvest periods (Tab. 3). Among the phenolic compounds analyzed, epicatechin (7.809 mg/100 g) was dominant in the fruits, followed by myricetin (2.632 mg/100 g FW), kaempferol (2.396 mg/100 g FW) and quercetin (0.655 mg/100 g FW). The lowest amount of phenolic compound was p-coumaric acid (0.006 mg/100 g FW), followed by cafeic acid and chlorogenic acid (0.013 and 0.043 mg/100 g FW, respectively) (Fig. 5). There are very limited studies, in which the phenolic compounds of figs were determined. Both epicatechin and catechin belong to the group of catechins and it is a very essential group of compounds in the Mediterranean diet [Auger et al. 2004]. In our study epicatechin content was predominantly higher than any other phenolic compounds. Likewise, Tawfik and Alhejy [2014] also obtained higher content of epicatechin (12.48 mg/100 g FW). Nakilcioglu and Hısıl [2013] also re-

Table 2. Phytochemical contents of 'Abbas' local fig variety

		Fig sampling dates					
Part	Parameters	First period	Second period	Third period	Fourth period	Period average	$D_{\%5\ periods}$
Pulp	Total phenolic (mgGAE/100 g)	156.02 ±17.38^{a}	137.05 ±1.85^{b}	125.25 ±0.85^{b}	124.50 ±4.22^{b}	135.71	16.96*
	Total flavonoid (mg/100 g)	445.52 ±50.76^{a}	152.80 ±5.08^{b}	68.77 ±13.54^{c}	85.86±8.08^{c}	188.241	50.27**
	DPPH (%)	17.31 ±4.12^{a}	14.12 ±3.09^{ab}	8.30 ±0.88^{b}	17.64 ±7.95^{a}	14.34	8.92
	Anthocyanin content (mg/L)	60.91 ±18.80^{ab}	78.96 ±14.29^{a}	40.60 ±1.50^{bc}	38.12 ±0.97^{c}	54.65	22.22*

Table 3. The content of individual phenolic compounds in 'Abbas' fresh fig local variety

Phenolic compounds (mg/100 g FW)	Fig sampling dates					
	First period	Second period	Third period	Fourth period	Period average	$D_{\%5\ periods}$
Caffeic acid	0.019 ±0.021	0.011 ±0.002	0.017 ±0.010	0.008 ±0.002	0.013	0.020
Catechin	0.699 ±0.023a	0.609 ±0.047a	0.606 ±0.058a	0.426 ±0.090b	0.590	0.092*
Myricetin	2.245 ±0.427	2.418 ±0.596	1.870 ±0.020	1.753 ±0.424	2.632	0.691
Kaempferol	2.337 ±0.246	2.396 ±0.274	2.356 ±0.250	2.497 ±0.075	2.396	0.415
Quercetin	0.760 ±0.204	0.546 ±0.207	0.703 ±0.174	0.612 ±0.168	0.655	0.345
Ferulic acid	0.315 ±0.034a	0.307 ±0.064a	0.117 ±0.044b	0.150 ±0.091b	0.222	0.115*
P-Coumaric acid	0.009 ±0.007	0.006 ±0.002	0.005 ±0.002	0.005 ±0.002	0.006	0.004
Gallic acid	0.150 ±0.015a	0.108 ±0.020b	0.118 ±0.005b	0.132 ±0.014ab	0.127	0.023*
Benzoic acid	0.537 ±0.035a	0.389 ±0.032b	0.400 ±0.046b	0.384 ±0.044b	0.427	0.069*
Syringic acid	2.651 ±0.433a	1.471 ±0.214b	2.553 ±0.067ab	2.595 ±0.322ab	2.317	0.530*
Epicatechin	8.880 ±0.307a	9.131 ±0.164a	7.133 ±0.717b	6.093 ±0.747c	7.809	1.014*
Ellagic acid	0.137 ±0.035	0.155 ±0.151	0.031 ±0.021	0.030 ±0.011	0.088	0.138
Naringenin	0.152 ±0.097a	0.023 ±0.030b	0.022 ±0.004b	0.020 ±0.000b	0.054	0.092*
Chlorogenic acid	0.026 ±0.012c	0.063 ±0.012a	0.037 ±0.007b	0.049 ±0.015ab	0.043	0.020*

*Different letters in the same row and column indicate significant differences ($p < 0.05$)
Range (mean ±standard deviation)

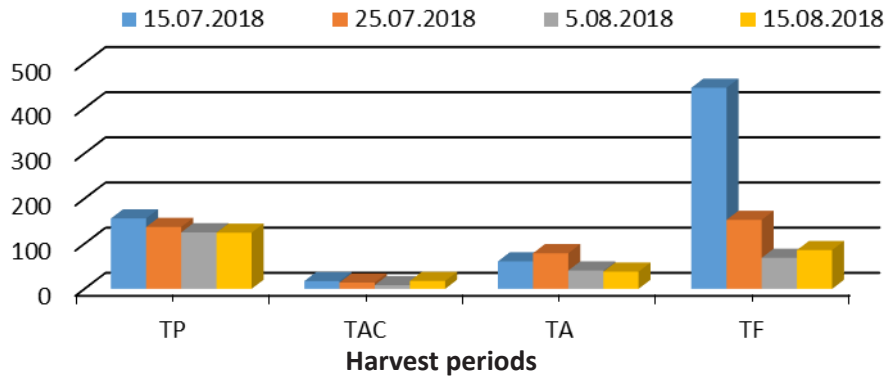

Fig. 4. Phytochemical change at different harvest times in 'Abbas' fig variety

ported that the highest amount of phenolic compound present in 'Sarılop' cultivar of fig was epicatechin and the lowest was syringic acid, which are in full agreement with our findings. In a study conducted on the figs growing in the coastal region of Slovenia, the most abundant phenolic compound was routine (4.89–28.7 mg/100 g FW), followed by catechin (1.07–4.03 mg/100 g FW), chlorogenic acid (0.46–1.71 mg/100 g FW), epicatechin (0.34–0.97 mg/100 g FW), gallic acid (0.14–0.38 mg/100 g FW), and syringic acid (0.022–0.104 mg/100 g FW) [Veberic et al. 2008]. The qualitative or quantitative variations could be attributed most likely to the differences in season, agricultural land type, and cultivar used. The TP content ranged from 124.50 to 156.02 (135.71 mg) mg GAE/100 g FW; TF ranged from 68.77 to 455.52 (188.20) mg/100 g

FW; the amount of TAC ranged from 8.30 to 17.64 (14.34) DPPH%; and the TA ranged from 34.12 to 78.96 (54.65) mg 100 g^{-1} FW (Tab. 2; the values showed in parentheses are the mean average of corresponding range). The phenolic contents reported in the first harvest were highest and in the fourth harvest were the lowest ones. This indicates that phytochemical contents were higher especially during the first harvest period and then gradually decreased in the subsequent harvests (Fig. 4). In the current study, the TP contents were quite higher than those reported in the previous studies conducted for other fig cultivars; 69.7–145.1 mg/100 g GAE [Del Caro and Piga 2008], 56.0–74.9 mg GAE/100 g FW [Solomon et al. 2006], 28.6–211.9 mg/100 g GAE [Pande and Akoh 2010, Caliskan and Polat 2011, Slatnar et al. 2011]. On the contrary, higher values have also been reported by Djuric et al. [2014] (up to 536.4 mg GAE/100 g FW) and Kamiloglu and Capanoglu [2015] (193–417 mg GAE/100 g FW) (Tab. 1). The TF values obtained were also higher than the earlier findings of Solomon et al. [2006] (45.6 mg/100 g), Kamiloglu and Capanoglu [2015] (44 mg/100 g) and Hoxha et al. [2015] (8.31–36.95 mg/100 g). However, Al-Farsi et al. [2005] reported the higher levels of TF than those reported by us. The total antioxidant capacity (TAC) reported in our findings is on par with Caliskan and Polat [2011]; conversely, the TACs reported by Solomon et al. [2006] and Veberic et al. [2008] are higher. In our study, the TA values were similar to those obtained from commercial fig varieties by Duenas et al. [2008] (31.79–96.81 mg/100 g) and Solomon et al. [2006] (30–100.9 mg/100 g), however, Caliskan and Polat [2012] (22.39–220.44 mg/100 g) and Hoxha and Kongoli [2016] (135.09–160.76 mg/100 g) reported relatively higher TA values than ours. Furthermore, our TA values were found to be higher than other commercial fig varieties as reported by Pourghayoumi et al. [2017] (0.8–4.44 mg/100 g) and Hoxha et al. [2015] (0.0–5.32 mg/100 g) (Tab. 2). It is evident from the current findings that the total phenolics, total anthocyanins, and total antioxidant capacities of some varieties in our study can be higher than other fig varieties in different countries. These findings indicate that total phenols and total anthocyanins are the main factors that affect the total antioxidant capacity in figs.

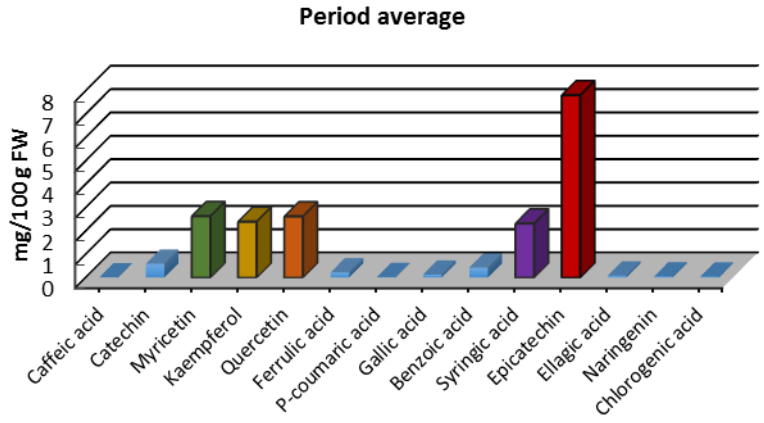

Fig. 5. Change in phenolic compounds in fig variety

CONCLUSIONS

To best of our knowledge, this is the first comprehensive study aimed to determine the phenolics, flavonoids, anthocyanin profile, and antioxidant activities in 'Abbas' variety of figs grown in Kahramanmaras ecological conditions. In this study, huge phytochemical variations were reported in the fig variety in a harvest

time-dependent manner. The variety evaluated in this study showed relatively higher levels of TP, TF, TAC, and TA than the other cultivars. There could be established a positive correlation between the phenolic contents and antioxidant activities of the samples. Therefore, the extracts of 'Abbas' variety can be used as a natural and easily accessible source of antioxidants. Its pulp happens to be an excellent foodstuff and can be used as a promising nutritional supplement and a good source of antioxidants. This study provides important information for the producers as well as the consumers toward increasing fig productivity and consumption by creating awareness about the health benefits of figs in a diet. The identification and characterization of phytochemical compounds in important varieties will also pave the way for the quality production of figs in Turkey, which would also significantly contribute to the breeding and crop improvement programs.

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