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Variation of Composition of Phenolic Compounds in the Apricot (*Prunus armeniaca* L.) Leaves by Seasons

Yilmaz Ugur[1], Selim Erdogan[2*], Ismet Yilmaz[3], Serkan Basgel[4]

[1]*Ministry of Food, Agriculture and Animal Husbandry, Apricot Research Institute Directorate, Malatya, Turkey*

[2]*Inonu University, Department of Analytical Chemistry, 44280, Malatya, Turkey*

[3]*Inonu University, Department of Pharmacology, 44280, Malatya, Turkey*

[4]*Central Mediterranean Regional Directorate of Customs and Trade Laboratory, 33100, Mersin, Turkey*

ABSTRACT

This study was aimed to determine qualitative and quantitative changes of the composition of phenolic compounds in leaf of five apricots (Prunus armeniaca L.) cv. by season.

These apricot varieties are "Hacihaliloglu" (HH), "Hasanbey" (HB) "Kabaasi" (KB) "Ismailaga" (IA) and "Cekirge" (CG) which are cultivar in Malatya province of Turkey. Sampling process was carried out in three different seasons being as April (Spring), June (Summer) and November (Autumn) corresponding by season. HPLC method was used for the determination of phenolic compounds in the samples. The results showed that, the most common phenolic compounds species in apricot leaves were chlorogenic acid, rutin, -(-) catechin and naringin. At the end of third period, while rutin and catechin contents were increased, chlorogenic acid and naringin were exponentially decreased.

As a result, Chlorogenic acid level of HH leaves were 1.7, 1.6, 1.5 and 1.3 times higher than that of KB, CG, IA and HB leaves in April, respectively. The chlorogenic acid in HH, CG and KB leaves according by seasons decreased by 43.81, 48.15, 52.50% from April to November, respectively. Rutin levels of in all leaves showed the highest increase with 83.56% in IA varieties. It was determined that there was an increase of HH 77.33%, HB 66.66% and KB 63.22% in apricot leaves.

Keywords: Apricot leaves, Polyphenol, Growing season, HPLC

INTRODUCTION

Plants, which are a potent source of antioxidants and these prevent the oxidative stress caused by oxygen and photons too. The term "plant phenolics" encompasses simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans and lignins. Phenolic components have been associated with diverse functions, including nutrient absorption, protein synthesis, enzyme activity, photosynthesis, structural components and allelopathy [1]. Phenolic compounds have been received much attention for their beneficial effects and they attributed to their donating electrons, scavenging free radicals, and reducing power abilities. In addition, natural antioxidants have the capacity to improve food quality and stability and can also act as Nutraceuticals to terminate free radical chain reactions in biological systems, and thus may provide additional health benefits to consumers [2,3]. Several thousands of natural polyphenols have been identified in plants, and many of them are consumed as foods. Although a more limited number are significant levels in most human diets. Dietary intake of phenolics is estimated to be approximately one gram per day. This is significantly higher than that of all other dietary antioxidants, including vitamin C, and E and carotenoids [4]. They are secondary metabolites of plants and generally involved in the defence against ultraviolet radiation or aggression by pathogens [5]. With the increased nutrient uptake, which is

rich in natural antioxidants is thought to decrease the risk of degenerative diseases [6,7]. The nutritional and/or therapeutic properties are related to the high level of phenolics as flavonoids, phenolic acids, glycosides, gallotannins, ellagitannins and proanthocyanidins and also these phenolics have an importance in fruit consumption [8]. Therefore, interest in antioxidant compounds has increased in recent years.

When the literature is examined, it is seen that many fruit species are rich in phenolic components. Besides, there are considerable amounts of phenolic compounds in the leaves of some fruit species. The researchers are better known that, fruits are rich a lot of phenolics from resveratrol (grape) to amygdalin (almond), from phenolic acids (apricot) to proanthocyanidin (cranberry, hawthorn). And also fruit leaves are sourced a lot of different kind antioxidant compounds (as chlorogenic and catechin; apricot, procyanidins and flavonoids; hawthorn, resveratrol, flavonoids, quercetin, rutin, kaempherol and naringin; grape leaves) [9,10]. In one study, Mountain hawthorn leaves were found to be rich in chlorgenic acid, rutin and quercetin [11]. Another study has shown that wolfberry leaves are rich in chlorgenic acid [12]. In a study on walnut leaves, it was found that ellagic acid, myricetin, catechin and rutin compounds were found to have a significantly higher concentration [5]. Another study found that catechin, epigallocatechin gallate, rutin, ferulic acid, caffeic acid and chlorgenic acid species were found in remarkable concentrations in blackberry and raspberry leaves [13].

Apricot production is one of the most important activities in total world fruit production. It is an important source for increase farmers profit in agricultural economic conditions of Turkey. Located at quite suitable conditions in terms of crop production considering, its climate and field conditions of Turkey has ranked first in the world with 811,609 tons of apricot production in 2013 by 16.69% [14].

The climatic conditions and soil structure of Malatya are not only suitable for apricot cultivation, but also a lot of other fruits such as plum, pear, hawthorn, grape, pomegranate, apple, cherry, cranberry, walnut and almond. It is well known by everyone who is interested that, "*Prunus armeniaca* L. is one of the most common varieties of the Rosaceae family, which is widespread in the northern hemisphere". Raj et al. [15] were reported that, the hepatoprotective effect of apricot leaf extracts against paracetamol induced liver toxicity in rats, and they were confirmed by liver tissue histopathological examinations. The presence of phenolic compounds in the apricot is an indisputable fact, as it is in plants and fruits of most species.

We found no data on qualitative and quantitative composition of phenolic compounds of apricot leaves by season. Therefore, we aimed to determine qualitative and quantitative analysis of phenolic compounds by using HPLC method apricot leaf samples during by season (from April to November).

MATERIALS AND METHODS

Collection of samples

The names of selected apricot varieties are "Hacihaliloglu" (HH), "Hasanbey" (HB) "Kabaasi" (KB) "Ismailaga" (IA) and "Cekirge" (CG) which have grown approximately 90% among all the apricot cultivars in Malatya.

In this study, the leave samples are collected from gardens of the Apricot Research Institute in Malatya Province and these were used, and sampling process was carried out in: April (Spring), June (Summer) and November (Autumn) (by season).

Instrumentation

Pressurized liquid extraction (PLE) was performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. The ASE 200 system with 11 ml stainless steel vessels for the pressurized liquid extraction was used for the extraction of polyphenols from the samples.

Reagents and standard solutions

Chlorogenic acid, rutin, caffeic acid, p-coumaric acid, naringin and gallic acid, –(-) catechin, quercetin, myricetin, –(-)epigallocatechin gallate, –(-) epicatechin gallates, –(-) epicatechin, –(-) gallocatechin,–(-) epigallocatechin, kaempferol and luteolin were purchased from Sigma (Sigma Chemical Co., Steinheim, Germany). Ethanol and ter-butylhydroquinone (tBHQ) were obtained from Merck (Darmstadt, Germany). All chemicals are of HPLC and analytical grade. High-purity water obtained from a Milli-Q system, Millipore (Bedford, MA, USA) was used for preparing all solutions. Glassware and other equipment's were carefully cleaned starting with 1-2% HNO_3 and ending with repeated rinsing with distilled, deionised water to prevent contamination.

Extraction of polyphenols

For extraction, temperature, pressure, static extraction time and solvent composition, were optimised.

For the analysis of phenolics of components from apricot leaves; the most suitable extraction method was determined. In this sense, prior to the extraction of polyphenols from different samples, operational parameters of the pressure liquid extraction (PLE, ASE-200) technique were established. Extraction procedure was performed under the conditions of 1500 psi pressure, 60˚C temperature, ethanol:water (80:20, v/v) extraction solution and 60 min static extraction time (Table 1).

Table 1: Optimized conditions for extraction of polyphenols from apricot leaf samples

Extraction name	Extraction
Extraction solvent	Ethanol:Water (80:20%, (including 0.1 g TBHQ)
Temperature (°C)	60
Pressure (psi)	1500
Heat-up time (min)	5
Extraction time (min)	20
Flush volume (%)	60
Number of cycle	3
Purge times (min)	1
Cell volume (ml)	11
Total extraction time (min)	60
Total solvent used (ml)	23

A sample of 1.00 ± 0.001 g dried apricot leaves was placed in 11 mL stainless steel extraction cell and the cell was placed in the carousel. Then the samples were extracted with about 23 ml of the solvent examined. The solvent was previously degassed to avoid the oxidation of the analytes under the operating conditions. Each of the analysis was examined with three replicates. Once the extraction finished, the extracts were filtered with 0.25 mm nylon membrane filter (Supelco Inc., Bellefonte, PA, USA) and solvent was evaporated to dryness in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) under nitrogen stream in a water bath at 40°C. The residue was reconstituted in (2 mL) ethanol-water (50:50, v/v) and filtered through a 0.45 µm PTFE filter (Waters, Milford, CA, USA) prior to injection into the HPLC system. Polyphenol concentrations in all the extracts were analyzed with HPLC.

HPLC analysis

Phenol analysis were performed on an Agilent Series 1100 liquid chromatography, equipped with a vacuum degasser, a quaternary pump and Agilent 1100 G 1315B DAD detector, connected to a HP Chem Station software. A reversed-phase ACE 5 C18 A11608 (250 × 4.6 mm, 4 µm) column was used. For gradient elution mobile phase, solution A contained 3% acetic acid in water; solution B contained mixture of 3% acetic acid, 25% acetonitrile and 72% water. The following gradient was used: 0-40 min, from 100% A to 30% A, flow rate 1 mL/min; 40-45 min, from 30% A, 70% B to 20% A, 80% B with flow rate 1 mL/min; 45-55 min, from 20% A, 80% B to 15% A, 85% B with flow rate 1.2 mL/min; 55-57 min, from 15% A, 85% B to 10% A, 90% B with flow rate 1.2 mL/min; and 57-75 min 10% A, 90% B with flow rate 1.2 mL/min. Operating conditions were as follows: column temperature, 30°C and injection volume, 20 µL. Detection was performed with UV-DAD at 280 nm.

A 1000 µg ml^{-1} stock solution of polyphenol was prepared by accurately weighing 1.0 mg or 1 ml of the standards, dissolving it in a beaker and then the powder was dissolved completely, the solution was quantitatively transferred into an amber volumetric flask and stored at 4°C away from light. Polyphenol standard solutions were always prepared daily by appropriate dilution of stock polyphenol standard solution (Merck) containing 1 mg ml^{-1}

polyphenols. All solutions for the analytical curve were prepared from this intermediary solution at the following concentrations: 0.1, 1.0, 2.0, 5.0, 10.0, 30.0 and 40.0 mg l^{-1} in water and or methanol.

Table 2: Analytical characteristics of polyphenol which analyzed by HPLC-DAD detection (n=3); Data presented as mean ± (SD)

Concentration Range (mg/l)	Phenolic acid	a	b	r	LOD	LOQ
1-100	Chlorogenic acid	7.3392 ± 0.033	-1.2585 ± 0.05	0.997	2.2×10^{-2}	6.8×10^{-2}
1-100	Rutin	8.5890 ± 0.61	-2.3247 ± 0.10	1	4.0×10^{-2}	1.2×10^{-1}
1-100	Naringin	19.1104 ± 1.26	6.7612 ± 0.31	0.999	3.6×10^{-2}	1.10×10^{-1}
0,2-20	Catechin	12.4836 ± 0.32	-1.3617 ± 0.02	0.999	5.3×10^{-3}	1.6×10^{-2}

Statistical analysis

Experimental dates were evaluated using analysis of variance (ANOVA) and significant differences among the means of three replicates ($p<0.05$).

RESULTS AND DISCUSSION

In order to ensure the linearity of the relationship between peak area and concentration, six standard solutions were prepared and suitably diluted with methanol-water. Standard solutions containing different concentrations of selected polyphenols, listed in the experimental section, were injected in the HPLC column and peak areas were determined at 280. The relationship between the concentration and the peak area is shown by a, b and r values were given in Table 2, where a and b are coefficients of the regression equation:

$y=ax+b$

with x being the concentration of the phenolic compound (ppm), y the peak area and r the correlation coefficient of the equation. All of the phenolic compounds showed good linearity and obeyed Beer's Law in the investigated concentration ranges of 0.2-20 mg/l and 1-100 mg/l. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by multiplying 3.3 and 10 with s/a, respectively, where s is the standard deviation of blank and a is the slope of the regression equation. The calibration parameters of phenolic acids with their standard deviations, correlation coefficient r, LOD and LOQ values are given in Table 2. Since the results of the repeatability and linearity are reliable for the purpose of determination, the used gradient elution method allowed a good separation of the phenolic compounds present with values of a above 1.0 in all cases (Figure 1). This method enabled identification of the phenolic compounds in all samples. The precisions of the analysis were calculated via peak area measurements and were given as standard deviation for a minimum three repeated runs.

Plants (leaves, flowers and fruits) are capable of synthesizing thousands of primary and secondary metabolites with diverse biological properties and functions. These compounds play a vital role in the plant life cycle by providing chemical cues to animals, pollinators, and seed disseminators that ensure plant reproductive and evolutionary success [16]. All criteria examined in this study were changed depending on the cultivars and sampling months. It was determined that chlorogenic acid, rutin, catechin and naringin are available in apricot leaves.

In the present study, the highest level of chlorogenic acid was measured as 167.75 ± 1.61 in HH at April and lowest level was measured as 59.85 ± 1.12 in HB at November. The highest level of rutin was measured as 146.48 ± 2.26 in İA at November and lowest level was measured as 24.78 ± 1.79 in IA at April. The highest level of catechin was measured as 12.62 ± 0.69 in HH at November and lowest level was measured as 6.79 ± 0.39 in HB at November. The highest level of naringin was measured as 57.62 ± 1.34 in ÇG at July and lowest level was measured as 13.71 ± 0.91 in same species at November (Table 3).

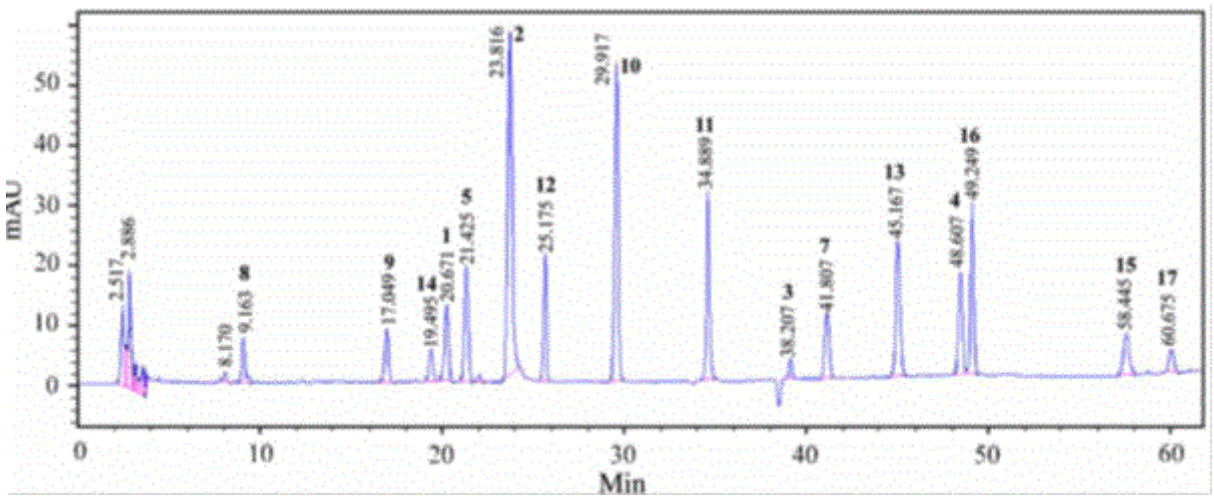

Figure 1: HPLC chromatogram of the polyphenol standard solution at 280 nm. 1, chlorogenic acid; 2, caffeic acid; 3, rutin; 4, resveratrol; 5, naringin; 6, epigallocatechin gallate; 7, gallic acid; 8, gallocatechin; 9, epigallocatechin; 10, p-coumaric acid; 11, epicatechin gallate; 12, epicatechin; 13, myricetin; 14, catechin; 15, quercetin; 16, phloridzin; 17, luteolin

Table 3: Identified and quantified phenolics of five apricot leaves extracted by PLE (mg/100 g dried sample); ND*: Non Detectable

Apricot species↓	**Polyphenols →**	**Chlorogenic**	**Rutin**	**Catechin**	**Naringin**
	Periods ↓	**(mg kg^{-1})**	**(mg kg^{-1})**	**(mg kg^{-1})**	**(mg kg^{-1})**
Hacıhaliloglu	April	167.75 ± 1.61	29.33 ± 0.56	ND*	ND*
	July	104.99 ± 1,03	29.02 ± 0.78	ND*	ND*
	November	94.25 ± 0.71	129.42 ± 0.70	12.62 ± 0.69	ND*
Kabaasi	April	101.23 ± 1.98	44.31 ± 0.49	ND	ND
	July	107.47 ± 0.95	50.59 ± 1.23	ND	ND
	November	105.61 ± 0.86	121.29 ± 1.95	ND	ND
Cekirge	April	117.86 ± 2.14	40.18 ± 1.21	ND	48.65 ± 1.57
	July	81.12 ± 1.46	46.06 ± 1.32	ND	57.62 ± 1.34
	November	60.66 ± 1.91	53.39 ± 1.92	ND	13.71 ± 0.91
Ismailaga	April	111.44 ± 1.73	24.78 ± 1.79	ND	ND
	July	92.78 ± 1.28	35.88 ± 1.99	ND	ND
	November	91.94 ± 2.47	146.48 ± 2.26	ND	ND
Hasanbey	April	126.51 ± 1.40	29.57 ± 0.79	ND	ND
	July	76.38 ± 0.4	51.27 ± 0.99	ND	ND
	November	59.85 ± 1.12	87.78 ± 0.46	6.79 ± 0.39	ND

The content changes of these substances were given in Table 3 are well associated by seasons of apricot leaves mentioned above. At the nascent stage of the plants, these compounds are considered to regulate plant growth and development, thus their high level indicated their important functions in growth periods. These compounds, especially chlorogenic acid and rutin, reached peak values in April and November, respectively, which are corresponded well with the growth periods of apricot trees. Temperature and light conditions have been changed in autumn, leaves turn yellow and anthocyanins in the leaves decompose or are transferred into storage organs, such as the bark or seeds before the leaves fall, and this was reflected by the low some polyphenol contents in autumn.

These compounds were identified from authentic standards and previous reports. Neo-chlorogenic acid and chlorogenic acid were previously identified in apricot peel extract and unpeeled and peeled canned apricot fruit [17-21]. Besides, researchers [22] showed that chlorogenic acids were the dominant polyphenols during apricot fruit ripening. The increase in the chlorogenic acid in the apricot leaves may be due to the heat stress, which increased during maturation, it was reported that chlorogenic acid is the major phenolic compound in the raw apricots [23-25].

The reason may be supposed that plants generate polyphenols to protect themselves against some stresses. In one study, it was reported that phenolic compounds contribute to the resistance of plants to physical stress that is caused by the injuries that take place during mechanized harvesting or due to insect bites and biological stress resulting from being infected by fungi, bacteria and viruses [26-27].

The changes in the polyphenolic compounds in the leaves during maturation may be correlated with fruit development and thus should be considered in apricot agriculture. Variation in these compounds may occur due to the variety, climate, soil and environmental conditions.

While rutin levels were shown an increase from april to november in five apricot cultivars (Table 3), the chlorogenic levels were shown a decrease in KB, HH, ÇG and HB varieties but it was shown a slight increase in İA specie from april to november (Table 3). Interestingly, catechin levels were measured only in HH (as highest), and in HB (as lowest) and both were at November (Table 3). The more interesting one, the naringin levels were measurable only CG species (Table 3). It may be surely say that, phenolic contents of apricot leaves were differentiated by varieties and seasons.

CONCLUSION

In conclusion, apricot leaves were collected at three maturation stages, and HPLC-DAD analyses revealed seventeen polyphenolic compounds in the apricot leaves. The major phenolic compounds were chlorogenic acid and rutin. Other phenolic compounds included catechin and its derivatives, naringin and its derivatives, and also caffeic acid. Qualitatively and quantitatively significant changes were observed in the polyphenolic compounds by season.

While rutin, catechin and naringin were found the highest levels as flavonoids in apricot leaves, the most abundant phenolic acid was chlorogenic acid. At November, chlorogenic acid and naringin contents are exponentially decreased; however, rutin and catechin are increased. During the growth season catechin and naringin was not detected in some leave samples. The samples of dried apricot leaves showed higher the values of chlorogenic acid (Table 3). The naringin was determined only in the samples of CG apricot leaves (Table 3).

Chlorogenic acid level of HH apricot leaves was 1.7, 1.6, 1.5 and 1.3 times higher than that of KB, CG, IA and HB leaves in April, respectively. The chlorogenic acid in HH, CG and KB leaves according to the growing seasons decreased by 43.81, 48.15, 52.50% from April to November, respectively. Rutin in the growing seasons of all leaves showed the highest increase with 83.56% in IA varieties. It was found that there was an increase of HH 77.33%, HB 66.66% and KB 63.22% in apricot leaves.

Herewith; data on the differences in phytochemical composition of apricot leaves material in the course of various vegetation stages can be used for rational planning of the harvesting of raw plant material rich in phenolic compounds with specific biological effects.

In conclusion, apricot leaves are a good source of polyphenolic compounds and the changes in these compounds may be affect fruit development and thus should be considered for the best agriculture practice of this plant. In this content, in the future, we plan to study for determination of some pharmacological activity of apricot leave extracts.

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