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Prunus armeniaca L. cv. Hacihaliloglu Fruits Extracts Prevent Lipid Peroxidation and Protect the Unsaturated Fatty Acids in the Fenton Reagent Environment

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This study investigated protective effects of the *Prunus armeniaca* L. cv. Hacihaliloglu fruits extract on the unsaturated fatty acids and the prevention of lipid peroxidation formation in the Fenton reagent environment. Lipid peroxidation level in the Fenton R group significantly high compared to control group, but lipid peroxidation in the apricot fruit extract groups significantly decreased according to the Fenton R group ($p < 0.0001$). In addition, it was determined that apricot extracts had scavenging effect on the DPPH (α,α-diphenyl-β-picrylhydrazyl) radical depending on the increase in flavonoid concentration. The fatty acid levels in the Fenton R group were lower than the control group ($p < 0.001$). However, fatty acid levels in the apricot fruit extract groups were higher than the Fenton R group ($p < 0.001$). Present results confirm that the apricot fruit extracts decrease lipid peroxidation level in the Fenton reagent environment and protects markedly unsaturated fatty acids in the environment with radical sourced oxidations. The examined apricot varieties were found to contain fructose, glucose, sucrose, β-sitosterol, α-tocopherol, stigmasterol, vitamin D, vitamin K, ergosterol and polyphenols such as catechin, rutin, resveratrol and myricetin.

Key Words: Apricot, Lipid peroxidation, Fatty acids, Flavonoids, α,α-Diphenyl-β-picrylhydrazyl, (DPPH).

INTRODUCTION

Sugar, organic acids, phenolic compounds and carotenoids play important roles in determining the nutritional value of many fruit and vegetables[1]. Clinical studies suggest that a diet which is high in fruit and vegetables reduces the incidence of cancer, coronary diseases and hypertension[2]. The most important benefit of fruit and vegetables in terms of human health is that they contain antioxidants. Free radicals cause the occurrence of some diseases, as well as enabling their progress, by causing lipid peroxidation, which is their most significant role. All types of biomolecules are negatively affected by free radicals. However, membrane lipids are the most delicate form of biomolecules and the most susceptible to damage[3].

Apricot is thought to be a rich food in terms of antioxidants because of the presence of flavonoids and carotenoids[4]. Although apricots grow widely, little is

known about their potential benefits in terms of human health. Several previous studies showed that apricot is rich in terms of vitamins A and C, polyphenols and β-carotene[5,6].

Turkey is one of the most important apricot-producing countries. According to 2005 data from the Food and Agriculture Organization (FAO), nearly 13 % (390,000 tons) of apricots were produced in Turkey. Malatya, which is situated in the east of Turkey, is an important center for apricot planting and production[7]. Even though many different apricot species were defined by several previous researchers[8,9], no detailed study was previously made of the chemical contents of Malatya apricots. The purpose of this study is to investigate the antioxidant activities and phytochemical characteristics of *Prunus armeniaca* L. cv. Hacihaliloglu. This species is planted widely in the Malatya region and represents 75 % of commercial apricot trees. The present study is of value because it is very important to be aware of the antioxidant effects of food in order to ensure good health and quality of life. Previous studies related to this subject generally dealt with the antioxidant capacities of vegetative resources and their chemical contents were not examined. In addition, this study also examined the phytochemical characteristics of apricot samples grown in the Malatya region of Turkey and investigated their impacts on human health by determining their antioxidant activities *in vitro*.

EXPERIMENTAL

Oleic acid (18:1, n 9), linoleic acid (18:2, n 6), linolenic acid (18:3, n 3), twin 20, tris-base and hydrochloride, quercetin, myricetin, resveratrol, cathechin, naringin, naringenin, kaempferol and HPLC grade methanol, acetonitrile, *n*-hexane, isopropyl alcohol, $FeCl_2 \cdot 2H_2O$, H_2O_2, KH_2PO_4, butylated hydroxytoluene (BHT), *n*-butanol, α,α-diphenyl-β-picrylhydrazyl, (DPPH), dimethyl sulphoxide (DMSO), 2-thiobarbituric acid (TBA) and ethyl alcohol were purchased from Sigma-Aldrich.

Apricot variety (*Prunus armeniaca* L.cv Hacihaliloglu) from different parts of the Malatya region (Akcadag, Darende and Malatya Center) were harvested directly from trees during the first and third weeks of July 2008. The fruits were immediately washed and frozen, to be further freeze-dried. Freeze-dried samples were maintained at -20 ºC prior to analysis. Only healthy looking fruits (without mechanical damage or bacterial infection) were selected for examination. All fruits were mature, with a declared place of origin (seller declaration). The antioxidant activity and flavonoid analysis were determined in DMSO extracts.

Preparation of apricot extracts: 50 g samples of fresh fruits were homogenized in 100 mL of 80 % methanol. Homogenates were centrifuged at 5,000 rpm for 5 min at 4 ºC. The supernatant was then concentrated by drying at under reduced 25 pressures at 50 ºC using a rotary evaporator. Each extract was re-suspended in DMSO to give a stock solution and stored at -20 ºC until analysis.

Antioxidative activity testing in the fatty acid environment: Antioxidative activities of the apricot extracts were determined by the method of Shimoi *et al.*[10]

with the following modifications: 1 mM $FeCl_2$ ($FeCl_2 \cdot 2H_2O$) and 3 μM hydrogen peroxide (H_2O_2) solutions were prepared freshly for every treatment, using doubly deionized water. Extracts of apricot fruits were also prepared freshly using DMSO. Methyl 3.97 mM oleate (18:1, n-9), 10.44 mM linoleate (18:2, n-6, LA) and 2.30 mM linolenate (18:3, n-3, LNA) were dissolved in the DMSO. Buffer solutions were prepared with 0.2 % Twin 20, 0.05M TRIS-HCl-BASE and 0.15M KCl (pH= 7.4).

During *in vitro* experiment, the first group was used as a control, the second group was Fenton reagent group, ($FeCl_2 + H_2O_2$, Fenton R) and the third group was Fenton R plus AH extract, the fourth group Fenton R plus DH extract and fifth group Fenton R plus MH extract

The first group was prepared and 0.4 mL fatty acid mixture (LNA: 3.26 μM/1 mL; 14.82 μM/1 mL LA and 4.99 μM/1 mL 18:1 methyl oleate) was suspended in 5 mL buffer solution. The second group was a Fenton reagent group and 0.4 mL fatty acid mixture 40 μM $FeCl_2$ and 60 μM H_2O_2 were suspended in 5 mL buffer solution. The third, fourth and fifth groups were Fenton reagent and apricot fruit extract and 0.4 mL fatty acid mixture 40 μM $FeCl_2$ and 60 μM H_2O_2 and 2 mL fruit extracts were suspended in 5 mL buffer solution.

All of the mixtures were incubated at 37 °C for 24 h. After incubation, 100 μL of 4 % (w/v) BHT solution was added to prevent further oxidation. Then, 1 mL of each mixture was taken and 1 mL 0.6 % TBA solution was added to the reaction mixture and incubated at 90 °C for 40 min. Samples were allowed to cool to room temperature and the pigment produced was extracted with 3 mL of *n*-butanol. Samples were then centrifuged at 6,000 rpm for 5 min and the concentration of the upper butanol layer was measured using a HPLC-fluorescence detector.

Quantitation of lipid peroxidation level *in vitro* environment: The products of peroxidation of fatty acids *in vitro* environment were determined by reading the fluorescence detector set at λ (excitation) = 515 nm and λ (emission) = 543 nm. Formation of the malonaldehyde (MDA) *in vitro* enviroment expressed as thiobarbituric acid-reactive substances (TBARS) calculated from a calibration curve using 1,1,3,3-tetraethoxypropane as the standard. The MDA-TBA complex was analyzed using the HPLC equipment. The equipment consisted of a pump (LC-10 ADVP), a UV-visible detector (SPD-10AVP), a column oven (CTO-10ASVP), an autosampler (SIL-10ADVP) a degasser unit (DGU-14A) and a computer system with class VP software (Shimadzu, Kyoto Japan). Inertsil ODS 3 column (15 × 4.6 mm, 5 μm) was used as the HPLC column. The column was eluted isocratically at 20 ºC with a 5 mM sodium phosphate buffer (pH=7.0) and acetonitrile (85:15, v/v) at a rate of 1 mL/min[11].

Quantitation of remaining fatty acids *in vitro* enviroment: Remaining mixtures of oleate, linoleate and linolenate in the test tube were converted to methyl esters by using 2 % sulfuric acid (v/v) in methanol[12]. Fatty acid methyl ester forms were extracted with *n*-hexane. Analysis was performed in a Shimadzu GC-17A V3

intstrument gas chromatograph equipped with a flame ionization detector (FID) and a 25 m, 0.25 mm i.d. Permabond fused-silica capillary column (Macherey-Nagel, Germany). The oven temperature was programmed between 160-215 °C, 4 °C/min. Injector and FID temperatures were 240 and 280 °C, respectively. The nitrogen carrier gas flow was 1 mL/min. The methyl esters of oleate, linoleate and linolenate were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Class GC 10 software version 2.01 was used to process the data. The results were expressed as μmol/mL.

Chromatographic conditions for flavonoid analysis: Chromatographic analysis was carried out using PREVAIL C_{18} reversed-phase column (15 × 4.6 mm) 5 μm diameter particles. The mobile phase was methanol/water/acetonitile (46/46/8, v/v/v) containing 1.0 % acetic acid[13]. This mobile phase was filtered through a 0.45 μm membrane filter (Millipore), then deaerated ultrasonically prior to use. Catechin (CA), naringin (NA), rutin (RU), resveratrol (RES), myricetin (MYR), morin (MOR), naringenin (NAR), quercetin (QU) and kaempferol (KA) were quantified by DAD following RPHPLC separation at 280 nm for catechin and naringin, 254 nm for rutin, myricetin, morin and quercetin, 306 nm for resveratrol and 265 nm for kaempferol. Flow rate and injection volume were 1.0 mL/min and l0 μL, respectively. The chromatographic peaks of the analyses were confirmed by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at a temperature of 25 °C.

Antioxidant assay by DPPH radical scavenging activity: The free radical scavenging effect in extracts was assessed by the decolouration of a methanolic solution of $DPPH^{\bullet}$ according to the method of Brand-Williams *et al.*[14]. A solution of 25 mg/L DPPH in methanol was prepared and 4.0 mL of this solution was mixed with 25, 50, 100, 250, 500 and 1000 μL of extract in DMSO. The reaction mixture was stored in darkness at room temperature for 0.5 h. The absorbance of the mixture was measured spectrophotometrically at 517 nm.

The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control - Abs sample)]/(Abs control)] × 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

Determination of sugars using liquid chromatography: Sugars in the combined extracts were determined using high-performance liquid chromatography (HPLC) with a refractive index detector (RID). The mobile phase was acetonitrile/water (75/25, v/v) and the elution was performed at a flow-rate of 1 mL/min. The temperature of the analytical column was kept at 40 °C. The column used was a supelcosil-NH_2, (25 × 4.6 mm, 5 μm, Sigma, USA). The analyses were performed in triplicate batches. Prior to the quantitative and qualitative determination of sugars in the sample, standard solutions were prepared of different sugars: sucrose, glucose and fructose. These standard solutions of different sugars were used to make calibration

lines for each of the sugars, which were later used for assessing the concentrations corresponding to the different peaks in the chromatograms.

HPLC Analysis of ADEK vitamins and Sterol amount: α-Tocopherol and sterols were extracted from the lipid extracts by the method of Sanchez-Machado[15] and Lopez-Cervantes *et al.*[16] with minor modifications. Five mL *n*-hexane/isopropyl alcohol mixture was treated 5 mL of KOH solution (0.5 M in methanol) were added and immediately vortexed for 20 s. The tubes were placed in a water bath at 80 °C for 15 min. Then after cooling in iced water, 1 mL of distilled water and 5 mL of hexane was added and the mixture was rapidly vortexed for 1 min, then centrifuged for 5 min at 5000 rpm. The supernatant phase were transferred to another test tube and dried under nitrogen. The residue was redissolved in 1 mL of the HPLC mobile phase [68:28:4 (v/v/v) methanol:acetonitrile:water]. Finally, an aliquot of 20 µL was injected into the HPLC column. Before injection, the extracts were maintained at -20 °C away from light.

Chromatographic analysis was performed using an analytical scale (15 cm × 0.45 cm I.D) Supelco LC 18™ column with a particle size 5 µm (Sigma, USA). HPLC conditions were as follows: mobile phase 60:38:2 (v/v/v): acetonitrile/ methanol/water; a flow rate of 1 mL/min; column temperature 30 °C. The detection was operated using two channels of a diode-array spectrophotometer, 326 nm for retinol and 202 nm for α-tocopherol and phytosterols[16].

Statistical analysis: Statistical analysis was performed using SPSS software (Ver 15.0). The experimental results were reported as mean ± SEM (standard error of means). Analysis of variance (ANOVA) and an LSD (least significant difference) test were used to compare the experimental groups with the controls.

RESULTS AND DISCUSSION

Lipid peroxidation (LPO): The LPO level was found to be significantly high in the group containing Fenton reagent ($p < 0.0001$) when compared to the control group (Fig. 1). When the groups containing apricot extracts and the control group were compared, a significant increase was observed in AH group and partial increases were observed in DH and MH groups ($p < 0.001$, $p < 0.05$). On the other hand, the MDA-TBARS level in the groups containing apricot fruit extracts was found to decrease significantly compared to the group containing Fenton reagent ($p < 0.0001$). When the groups containing apricot extracts were compared among each other, the LPO level in the DH and MH groups was observed to be lower than the AH group ($p < 0.0001$). No statistical difference was observed between the MH and DH groups (Fig. 1).

Parlakpinar *et al.*[4] examined the beneficial effects of apricot-based nutrition among rats with myocardial ischemia-reperfusion injury. It was observed that the rate of ischemia-reperfusion decreased more significantly among the rats in the apricot nutrition groups compared to the control group.

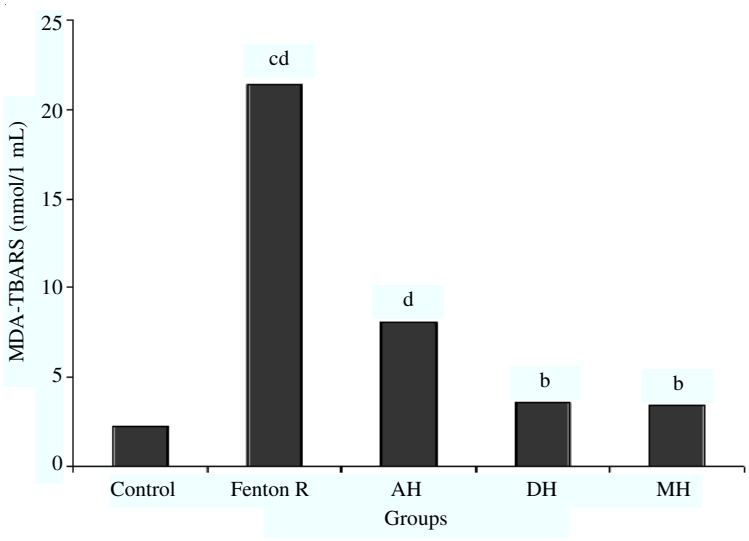
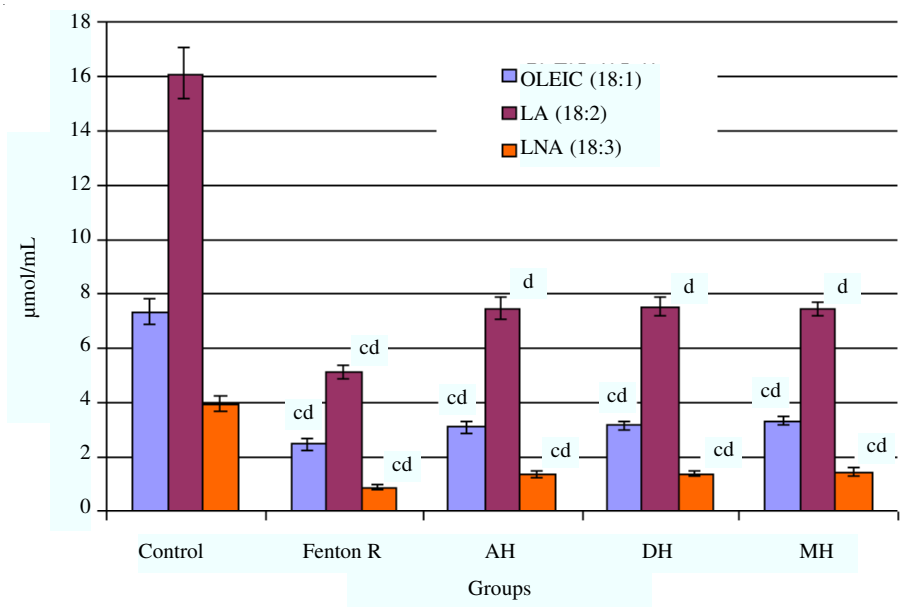


Fig. 1. *The levels of MDA-TBARS in the environments of Fenton reagent and Fenton reagent with apricot fruit extracts (Fenton R: Fenton reagent)
cd: $p < 0.0001$, d: $p < 0.001$, c: $p < 0.01$, b: $p < 0.05$, a: $p > 0.05$

Remaining fatty acid levels: When the LNA (18:3 n-3) amount in the reaction media was compared with the control group, significant decreases were observed in Fenton R and fruit extract groups ($p < 0.0001$) (Fig. 2). When the groups containing apricot extract were compared according to Fenton R group, it was found that LNA amount was significantly higher in AH, DH and MH groups ($p < 0.001$). In addition, no statistically significant difference was found between the groups containing apricot extract ($p > 0.05$). Linoleic acid (18:2 n-6) and oleic acid (18:1, n-9) amounts were observed to decrease in all groups when compared to the control group ($p < 0.0001$). When the groups containing apricot extracts were compared to the Fenton R group, amounts of both linoleic acid and oleic acid were found to be high ($p < 0.002$) (Fig. 2).

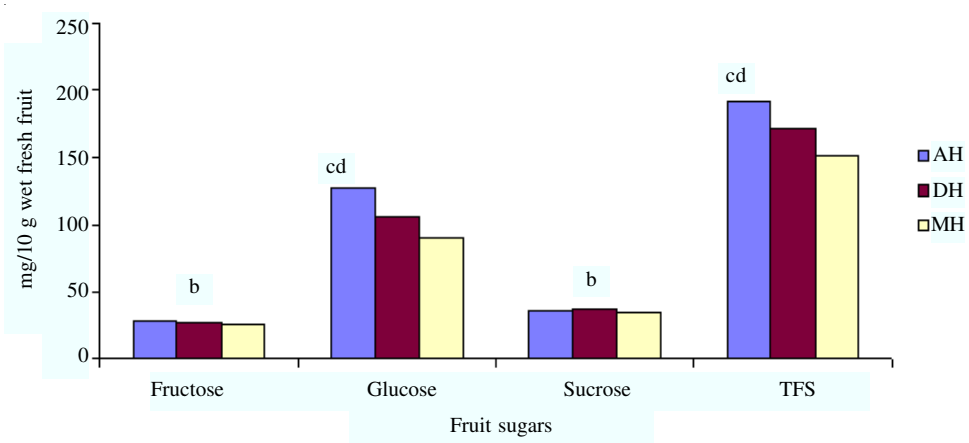
In the present study, when fatty acid quantity was examined each of the three fatty acids in *Prunus armeniaca* including extracts are high compared to Fenton reagent group. So it is presumed that the flavonoids of extracts may protect unsaturated fatty acids from radical sourced oxidations. All favonoids acted as antioxidants on oxidation of methyl linoleate although the antioxidant response of kaemferol and rutin is weak. Quercetin and myricetin inhibits the hydro peroxide formation in methyl linoleate environment[17].

Fruit sugar levels: Sugar analysis showed that fructose, glucose and sucrose were present in all apricot samples (Fig. 3). When the amounts of sugars were compared, it was found that glucose was the most common sugar type. On the other hand, when the apricot samples were compared, no statistically significant difference was found among the groups, although the amount of fructose in the AH



cd: $p < 0.0001$ d: $p < 0.001$ c: $p < 0.01$ b: $p < 0.05$ a: $p > 0.05$

Fig. 2. Levels of linolenic acid (18:3), linoleic acid (18:2, n-6) and oleic acid (18:1, n-9) in the reaction environment (µmol/1 mL)



cd: $p < 0.0001$ d: $p < 0.001$ c: $p < 0.01$ b: $p < 0.05$ a: $p > 0.05$

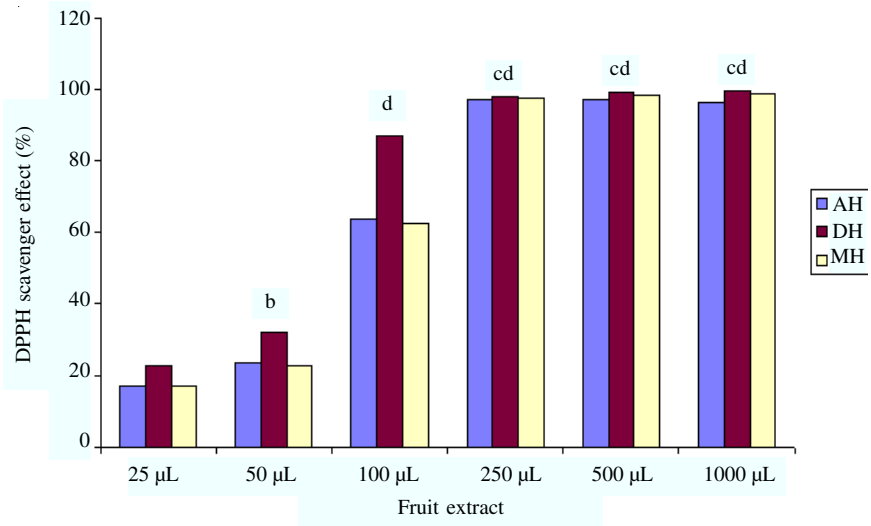
Fig. 3. Fruit sugar content (mg/10 g)

group was higher in comparison to the other groups ($p > 0.05$). However, when the comparison was made on the basis of glucose contents, the ranking was as such: AH > DH > MH (Fig. 3). In addition, although there was no difference among the groups in terms of sucrose contents, MH group was found to have relatively low contents of sucrose ($p < 0.05$).

Furthermore, when the groups containing apricot extracts were compared according TFS amounts, a significant difference was observed in the AH group when compared to the DH and MH groups ($p < 0.0001$) and this difference was found to be high between the AH group and MH group ($p < 0.001$) (Fig. 3).

Drogoudi *et al.*[18] examined the sugar, mineral and antioxidant contents of apricots. They reported that the most common sugar type in apricot fruit and its hybrids was sucrose and the other sugar types contained in apricots were glucose, sorbitol and fructose-inositol, respectively. According to the study by Akin *et al.*[19] Malatya apricot samples contained the sucrose, glucose and fructose.

Scavenging effects on the DPPH• radical: According to the results of DPPH free radical cleaning, all apricot samples were reported to display antioxidant activity from 100 µL concentration and this activity was observed to increase as the concentration increased (Fig. 4). The DH group was found to have more significant radical cleaning characteristics than the other groups in 100 µL concentration. When the groups were compared depending on - increasing concentration the group having the highest antioxidant capacity was found to be the DH group. On the other hand, when the concentrations were compared among each other, no statistical difference was reported between 25 and 50 µL, but the activity was observed to increase from 100 µL depending on the flavonoid concentration of the apricot extracts ($p < 0.0001$) (Fig. 4).



cd: $p < 0.0001$ d: $p < 0.001$ c: $p < 0.01$ b: $p < 0.05$ a: $p > 0.05$

Fig. 4. DPPH• scavenger effect of apricot fruit extracts (%)

Stratil *et al.*[20] studied the activities of apricot in their study which they conducted for determining the antioxidant activities and phenolic compounds of fruits. For determining antioxidant activity, they used the methods such as TEAC and FRAP

in addition to the DDPH method which was also used in the present study. Their results indicated that apricot fruit was high in total phenolic content. On the other hand, the data acquired using the DPPH method (although it is the same as TEAC method) were observed to be significantly low. It was concluded that this significant difference between the values stemmed from the high stability of DPPH radical.

Obviously there is a correlation between the results of this experiment and the results of MDA-TBA. It is also presumed that this may be related to flavonoid levels.

Vitamin and phytosterol levels: According to the results of the vitamin analysis, vitamin K_1, D, α-tocopherol and vitamin K_2 were observed in all apricot samples (Table-1). It was found that the amount of α-tocopherol was higher than other vitamins in all groups. The AH group contained particularly high levels of this vitamin, although no statistically significant difference was found among groups ($p < 0.001$, $p > 0.05$). It was also found that vitamin K_1 content was partially low in the AH group, but no statistically significant difference was found among the other groups ($p < 0.05$, $p > 0.05$). Moreover, the amount of vitamin K_2 was high in DH, MH and AH groups, respectively. When vitamin D contents were compared it was found that vitamin D amount was significantly high in AH group and that there was no difference among other groups ($p < 0.001$, $p > 0.05$).

TABLE-1
CONTENT OF LIPID SOLUBLE VITAMINS AND PHYTOSTEROLS IN APRICOT VARIETIES

Lipophilic vitamins and phytosterols	AH	DH	MH
Vitamin K_1	1.89±0.04 ^{b}	2.17±0.22	2.07±0.07
Vitamin D	1.02±0.07 ^{d}	0.60±0.02	0.61±0.04
α-Tocopherol	19.47±0.50 ^{c}	11.05±0.17	15.77±0.22
Vitamin K_2	0.22±0.04	0.43±0.03 ^{b}	0.36±0.06
β-Sitosterol	41.10±0.38	26.06±0.15 ^{cd}	46.05±0.72
Stigmasterol	19.82±0.25 ^{cd}	9.28±0.34	9.23±0.10
Ergosterol	0.79±0.04	1.02±0.03	5.20±0.22^{cd}

cd: $p < 0.0001$ d: $p < 0.001$ c: $p < 0.01$ b: $p < 0.05$.

According to the results of the phytosterol analysis, all groups containing apricot samples were found to include ergosterol, stigmasterol and β-sitoterol (Table-1). The most common phytosterol among all phytosterols was β-sitosterol. Statistical comparison of β-sitosterol contents indicated that DH group contained significantly low amounts of this fitosterol and there was no significant difference between other groups ($p < 0.001$, $p > 0.05$).

Among all the apricot groups, the ergosterol content was highest in the MH group ($p < 0.001$) and a partial statistical difference was observed among the other groups ($p < 0.01$). The AH group contained the highest stigmasterol content in terms of stigmasterol contents ($p < 0.0001$) whereas no difference was observed among the other groups ($p > 0.05$) (Table-1).

Turan *et al.*[21] investigated fatty acid, triacyglyceride, phytosterol and tocopherol contents in seed oil of apricots planted in the Malatya province of Turkey. They reported that γ-tocopherol and β-sitosterol were dominant in terms of tocopherols and phytosterols. Munzuroglu *et al.*[5] reported that levels of vitamin, β-carotene and selenium showed significant differences among species and among the same species planted in different regions. The highest levels of vitamin A and E and β-carotene were observed in Hacihaliloglu species when compared to other apricot species. In addition, they reported that apricot contained less vitamin E compared to other fruit and vegetables.

Flavonoids of apricot fruits: According to the results of flavonoid analysis, rutin and catechin flavonoids were high in all apricot samples whereas other flavonoids were present at lower levels (Table-2).

TABLE-2
FLAVONOIDS AND RESVERATROL CONTENT IN APRICOT VARIETY
(*Prunus armeniaca* L. cv. HACIHALILOGLU) (µg/1 g)

Flavonoids	AH	DH	MH
Cathechin	1314.66±4.61	891.33±37.29[cd]	1161.66±19.29
Rutin	422.83±3.17	566.66±2.25 [d]	374.50±1.50
Resveratrol	14.66±0.28	18.16±0.28 [b]	14.50±0
Myricetin	4.16±0.28	10.50±0.00 [d]	5.83±0.76
Morin	1.00±0.50	2.16±0.76 [d]	Trace
Naringenin	0.50±0.001	0.66±0.28 [c]	Trace
Quercetin	Trace	Trace	Trace
Kaempferol	Trace	Trace	Trace
Total	1758.81 [cd]	1489.47	1556.49

cd: $p < 0.0001$ d: $p < 0.001$ c: $p < 0.01$ b: $p < 0.05$.

It was found that the AH group contained a significant amount of catechin flavanoid and that there was no significant difference between DH and MH groups ($p < 0.001$, $p > 0.05$). Comparison of rutin and myricetin contents showed that the DH group had the highest content and a partially statistically significant difference was observed among other groups ($p < 0.001$, $p < 0.05$).

In addition, analysis showed the presence of resveratrol in all apricot groups (Table-2). Although no significant differences were observed between all groups in term of resveratrol levels, the DH group was found to contain slightly more resveratrol when compared to the other groups ($p < 0.05$).

When total phenolic compound levels were compared in accordance with the results, it was found that the AH group contained significantly high levels of phenolic compounds ($p < 0.0001$) and a partial difference was observed between DH and MH groups ($p < 0.05$) (Table-2).

As a result of the present study, apricot was observed to contain most flavonoids in terms of phenolic compounds. Among the flavonoid group, the presence of rutin and catechin was most notable. Some previous studies suggested that the phenolic compound of apricot was comprised of chlorogenic acid, neochlorogenic acid, rutin, catechin and epicatechin[8,9].

The analysis of Hacihaliloglu apricots used in present study indicated that in addition to flavonoids, this apricot contained a phenolic compound called resveratrol, which was reported to have significant antioxidant, antimutagenic, antiinflamamtory and carcinogenesis effects. However, this phenolic compound was not observed in the materials used in previous studies of apricot characteristics.

In conclusion, it was found that the Hacihaliloglu apricot species grown in the Malatya region contained phenolic compounds and vitamins in different amounts, depending on the location where the plant was grown. Phenolic compounds and vitamin content is closely related to the prevention of lipid peroxidation of apricots and reduced risk of chronic diseases. There may be some differences in the amounts and the diversity of these phytochemical characteristics contained in the fruit.

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