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Fruit Sugar, Flavonoid and Phytosterol Contents of Apricot Fruits (*Prunus armeniaca* L. cv. Kabaasi) and Antioxidant Effects in The Free Radicals Environment

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The aim of this study is to investigate the preventive effects of the *Prunus armeniaca* L. cv. Kabaasi fruits extract on the lipid peroxidation (LPO) formation in the Fenton reagent environment and analyze the flavonoid, sugar and vitamin contents. The LPO level in the Fenton R group was significantly higher than the control group ($p < 0.0001$), but its level in the fruit extract group was lower than the Fenton R group ($p < 0.001$). In addition, the LPO level in the fruit extract group was significantly decreased when the fruit extract amount was gradually increased ($p < 0.0001$). It was determined that apricot extracts had scavenging effect on the DPPH$^{\bullet}$ radical depending on the fruit extract amount. The examined apricot varieties were found to contain β-sitosterol, α-tocopherol, stigmasterol, vitamin D, Vitamin K, ergosterol and polyphenols such as catechin, rutin, resveratrol and myricetin. The results confirm that the apricot fruit extracts decrease LPO level in the Fenton reagent environment and have scavenging effect on the DPPH$^{\bullet}$ depending on extract level. The examined apricot varieties are seemed to be good sources biologically active compounds such as lypophilic vitamins and phytosterols.

Key Words: Apricot, Lipid peroxidation, α,α-Diphenyl-β-picryl-hydrazyl, Flavonoids, Fruit sugars, Phytosterol.

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is classified under the Prunus genius of Prunoidae sub-family of the Rosaceae family of the Rosales group. Apricot has an important place in human nutrition and can be used as fresh, dried or processed fruit. As known, the fruit of apricot is not only consumed fresh but also used to produce dried apricot, frozen apricot, jam, jelly, marmalade, pulp, juice, nectar, extrusion products, *etc*. Moreover, apricot kernel is used in the production of oils, benzaldehyde, cosmetics, active carbon and aroma perfume[1,2].

Turkey is the leading apricot producer of the world. According to the FAO statistical database in 2005, 390000 tons of apricot were produced in Turkey contributing to *ca.* 13 % of the total apricot production in the world[3]. Malatya region, of eastern Turkey is particularly important for cultivation, production and processing of apricots. Moreover, the apricots grown in this region has a reputation for their

characteristics and quality. The climate, structure and content of the soil and the other environmental conditions in Malatya region enable the production of quality apricots with dry matter and sugar content.

Apricot is thought to be a rich food in terms of antioxidants because of the flavonoids and carotenoids it contains[4]. However, even though apricots grow widely, little is known about their potential benefits in terms of human health.

The purpose of this study is to investigate the antioxidant, antiradical activities and phytochemical characteristics of *Prunus armeniaca* L. cv. Kabaasi. This species is planted widely in the Malatya region and represents 50 % 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 contrast to present studies, the aim of this study is to investigate the preventive effects of the *Prunus armeniaca* L. cv. Kabaasi fruits extract on the prevention of lipid peroxidation (LPO) formation that is arising from $OH^{\bullet}$ *in vitro* environment with $DPPH^{\bullet}$ radical scavenging effects.

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, Na_2HPO_4, 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.

Fruit materials of apricot varieties: Apricot varieties (*Prunus armeniaca* L.cv Kabaasi) from different parts of the Malatya region [Akcadag (AK), Darende (DK) and Malatya Center (MK)] 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.

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

Preventing effect on the LPO formation in the unsaturated fatty acid environment: Preventing effect on the LPO of the apricot extracts were determined by the method of Shimoi *et al.*[5] 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. 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 1st group was used as a control, the 2nd group was Fenton reagent group, ($FeCl_2 + H_2O_2$, Fenton R) and the 3rd group was Fenton R plus AK extract, the 4th group Fenton R plus DK extract and 5th group Fenton R plus MK extract.

The 1st 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) was suspended in 5 mL buffer solution. The second group was a Fenton R 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 3rd, 4th and 5th groups were Fenton R plus apricot fruit extracts and 0.4 mL fatty acid mixture 40 µM $FeCl_2$ and 60 µM H_2O_2 and 0.5 mL, 1 mL 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 pink colour 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 *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 AD_{VP}), a Fluorescence detector (RF-10_{XL}), a column oven (CTO-$10AS_{VP}$), an autosampler (SIL-$10AD_{VP}$) 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[6] at 20 ° C with a 5 mM sodium phosphate buffer (pH = 7.0) and acetonitrile (85:15, v/v) at a rate of 1 mL/min.

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[7]. 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 ºC.

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 according to the method of Brand-Williams *et al.*[8]. 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.

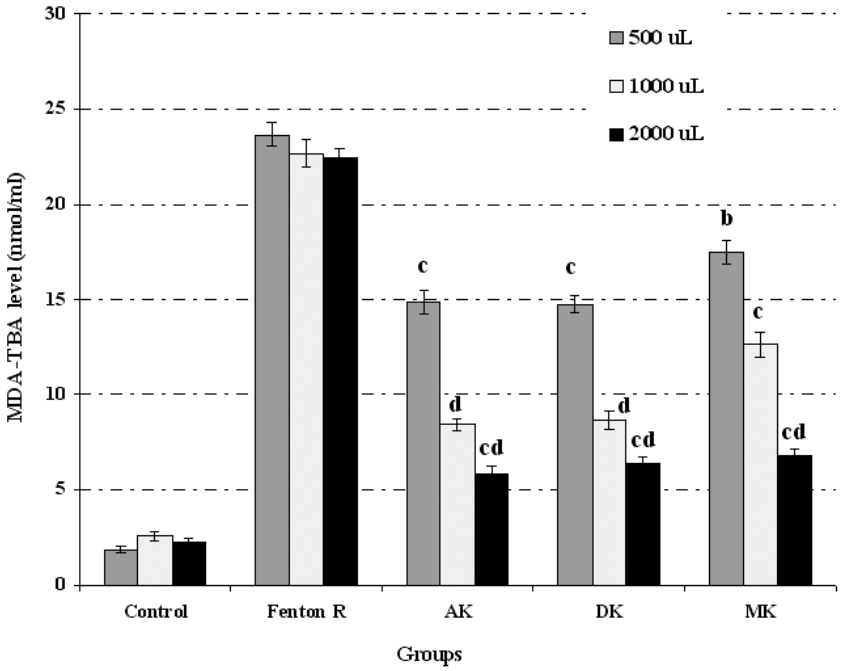
The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control - Abs sample)]/(Abs control)] × 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

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 was used 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.

Statistical analysis: Statistical analysis was performed using SPSS software (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 results: 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). In addition, when the groups containing apricot extracts and the control group were compared, a significant increase were observed in AK, DK and MK groups ($p < 0.001$). However, the MDA-TBA level in the groups containing apricot fruit extracts was found to decrease significantly compared to the group containing Fenton R ($p < 0.0001$).



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

Fig. 1. Levels of MDA-TBA of groups Fenton R and Fenton R with apricot fruit extracts

It was obtained that the LPO formation was decreased with increasing level of fruit extract. It was determined that the addition of 2 mL extract decreased more the LPO level as compared to the addition of 0.5 and 1.0 mL extracts. It was observed that while there were no significantly statistical differences between AK and DK varieties of 0.5, 1.0 and 2.0 mL additions, MK was found less preventive in the formation of LPO as compared to AK and DK for 0.5, 1 and 2 mL, (respectively, $p < 0.001$, $p < 0.05$) (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. In addition, a significant decrease was observed in LPO rates in the hearts of the apricot nutrition groups. There are similarities between LPO values of this *in vitro* study and the *in vivo* study of Parlakpinar *et al.*[4]. It can be concluded that this is because apricot varieties have similar antioxidant molecule contents.

Phenolic compounds are known as high level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals[9] they have prooxidant properties *in vitro* particularly in the presence of transition metal ions such as iron and copper[9,10]. This may be related to the ability of flavonoids to undergo autooxidation catalyzed

by transition metals to produce free radicals such as hydroxyl radicals *via* Fenton chemistry[11]. It is thought that despite its higher levels of flavonoid, apricot varieties showed a low performance in decreasing LPO level because of the above-mentioned reasons.

Scavenging effects on the DPPH• radical: According to the results of DPPH• free radical scavenging, 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. 2). The AK 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 AK group. On the other hand, when the concentrations were compared among each other, no statistical difference was reported between 25 µL 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. 2).

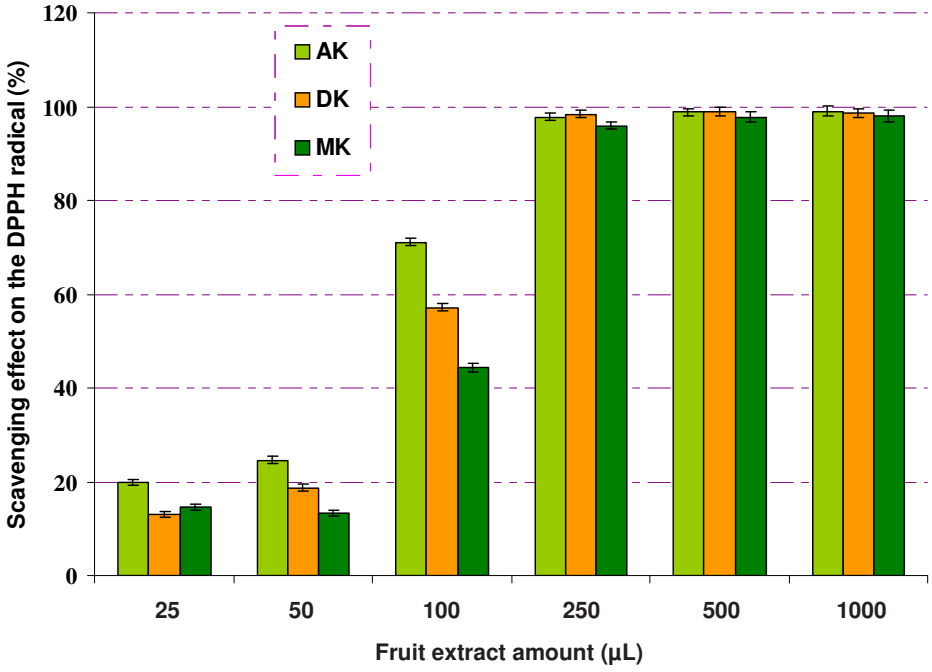


Fig. 2. DPPH• scavenger effect of apricot fruit extracts (%)

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

Stratil *et al.*[12] 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 DDPH which was also

used in the present study. Their results indicated that apricot fruit was high in total phenolic content.

Fruit sugar levels: Sugar analysis showed that fructose, glucose and sucrose were present in all apricot samples. In addition, trace level maltose were found in fruit extracts. 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 MK group was higher in comparison to the other groups ($p < 0.05$). However, when the comparison was made on the basis of glucose and sucrose contents, the ranking was as such: AK > MK > DK (Fig. 3). On the other hand sucrose content in DK group was lower than other apricot varieties ($p < 0.05$) (Fig. 3).

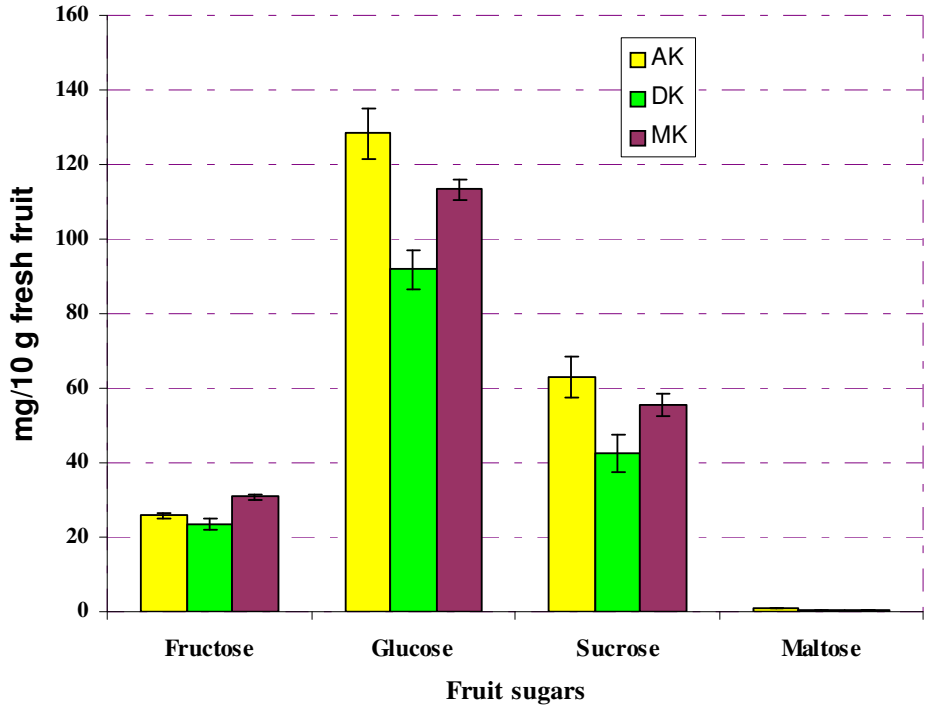


Fig. 3. Fruit sugar content (mg/10 g)

The consumer preference for apricots is greatly influenced by its sugar content, constituting an important compositional property[13,14]. Fruit sugar analysis showed that the commonest sugar type was glucose in apricot varieties of the present study. According to the study by Akin *et al.*[15], Malatya apricot samples contained the sugars like sucrose, glucose and fructose.

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. It was found that vitamin K_1 content was significantly high in the DK group and partial significant difference was found among the other groups ($p < 0.0001$, $p < 0.05$). When vitamin D contents were compared it was found that vitamin D amount was significantly high in DK group and that there was not a difference among other groups ($p < 0.001$, $p > 0.05$).

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.

Among all the apricot groups, the ergosterol content was highest in the AK group ($p < 0.001$) and a partial statistical difference was observed among the other groups ($p < 0.01$). The DK group contained the lowest stigmasterol content in terms of stigmasterol contents ($p < 0.0001$) and there was partial significant difference was observed among the other groups ($p < 0.001$) (Table-1).

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

Lipophilic vitamins and phytosterols	AK	DK	MK
Vitamin K_1	0.70 ± 0.02	5.00 ± 0.02[cd]	1.53 ± 0.08
Vitamin D	0.95 ± 0.02	1.45 ± 0.20[d]	0.17 ± 0.03
α-Tocopherol	9.30 ± 0.33	20.63 ± 0.63[d]	5.08 ± 0.17
β-sitosterol	21.08 ± 0.21	50.82 ± 0.85	11.90 ± 0.88[d]
Stigmasterol	8.27 ± 0.35	2.08 ± 0.07[cd]	5.11 ± 0.12
Ergosterol	6.07 ± 0.04[d]	1.43 ± 0.16	0.50 ± 0.02

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

Turan *et al.*[16] investigated fatty acid, triacylglyceride, 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.

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).

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. When total phenolic compound levels were compared in accordance with the results, it was found that the MK group contained significantly high levels of phenolic compounds ($p < 0.0001$) (Table-2).

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

Flavonoids	AK	DK	MK
Catechin	764.16 ± 5.68	973.33 ± 13.96	1109.33 ± 20.03[d]
Rutin	286.66 ± 3.05	366.33 ± 2.84	422.33 ± 9.81[d]
Resveratrol	20.00 ± 0	16.50 ± 0	21.83 ± 0.28
Myricetin	1.17 ± 2.02	3.17 ± 0.28	7.83 ± 0.76[d]
Morin	1.33 ± 0.28	Trace	2.5 ± 0.21
Kaempferol	Trace	1.5 ± 1.00	Trace
Total	1073.32	1360.83	1563.82[cd]

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

It has been previously found that the phenolic compound of apricot was comprised of chlorogenic acid, neochlorogenic acid, rutin, catechin and epicatechin[17]. There are many studies on the effects of individual flavonoids on lipid peroxidation[5,18,19] the information is defined to related combination of different flavonoids on lipid peroxidation.

In conclusion, it was found that the Kabaasi 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. This is not due to a single factor, but may be affected by sunlight, soil type, climatic differences and the location where the plant grows and differences between fruit species[17]. The amount of vitamins, minerals and sugar may vary from region to region because of the elements contained in the soil where the fruit grows[20]. Not only environmental factors, but also genetic factors, may mean that vitamin and mineral levels are different among the same apricot species and also in the same species in different cultural forms.

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