

## Antioxidative Activity and Phytochemical Composition of Dried Apricot

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### Abstract

In this study, the phytosterol and vitamin values of apricot fruits, which had been dried with different methods, and the antioxidant activity against iron (II) chloride (FeCl<sub>2</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induced *in vitro* hepatitis oxidation have been determined. In addition, antioxidant capacity of apricots was determined by using DPPH free radical. As a result, apricot extracts inhibited lipid peroxidation and protected amount of total protein. Additionally, DPPH free radical scavenging effect of the species showed a parallel increase in dose. We think that rich phytochemical compounds of apricot fruits (for example; phytosterols, flavonoids, polyphenols and vitamins) play a role in this effect.

**Keywords:** Apricot, Lipid peroxidation, Protein, DPPH, Vitamin, Phytosterol

## Kuru Kayısının Antioksidan Aktivitesi ve Fitokimyasal Kompozisyonu

### Özet

Bu çalışmada, farklı yöntemlerle kurutulmuş kayısı meyvesinin fitosterol ve vitamin seviyeleriyle, demir (II) klorür ve hidrojen peroksitle indüklenen *in vitro* karaciğer oksidasyonuna karşı antioksidan aktivitesi belirlendi. Ayrıca kayısının antioksidan kapasitesi DPPH serbest radikali kullanılarak belirlendi. Sonuçta, kayısı ekstraktları lipid peroksidasyonunu önledi ve total protein miktarını korudu. Buna ek olarak, türlerin artan doza paralel olarak DPPH serbest radikali temizleme etkisi görüldü. Biz bu etkinin kayısı meyvesindeki zengin fitokimyasal kompozisyonun (örneğin; fitosteroller, flavonoidler, polifenoller ve vitaminler) rol oynadığını düşünüyoruz.

**Anahtar kelimeler:** Kayısı, Lipid peroksidasyonu, Protein, DPPH, Vitamin, Fitosterol

### 1.Introduction

Oxidative stress appears to play an important role in several human chronic diseases, such as atherosclerosis and cancer [1]. Clinical studies suggest that a diet which is high in fruit and vegetables reduces the influence 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. Therefore, antioxidants may be useful in the prevention and treatment of these diseases. They help against the damaging effects of free radicals and preserve the oxidant-antioxidant balance [3].

Apricot (*Prunus armeniaca* L.) is classified under the *Prunus* species of the Rosaceae family of the Rosales group. This type of fruit is a cultivated type of zerdali (wild apricot) which is

produced by inoculation [4]. Apricot has an important place in human nutrition and can be used as fresh or dried fruit. Apricot is one of the most popular of the temperate tree fruit species, having a total world production of about 2.6 million tons, with Turkey (370,000 tons) as the leading country, Persia (285,000 tons) and Italy (244,000 tons) being the other main producers [5]. The most important cultivars of apricot grown in Malatya city in eastern Anatolia are classified as Hacıhaliloglu, Kabaasi, Hasanbey, Soganci and Cataloglu and this region is the biggest export center for dried type of apricot in Turkey as 80-85% of world's apricots are exported from here [6]. It is thought that apricot is a rich food in terms of antioxidants because it contains the flavonoids and carotenoids [7]. This is a rich source of potassium also.  $\beta$ -carotene,

which is the pioneer substance of vitamin A, is necessary for epithelia tissues covering our bodies and organs, eye-health, bone and teeth development and working of endocrine glands. Moreover, vitamin A plays important role in reproduction and growing functions of our bodies, in increasing body resistance against infections. However, even though apricots are produced widely, little is known about their potential benefits in terms of human health [8].

Apricots are harvested in July and either sun-dried without additives (sun dried, or locally known as *Gün Kurusu*) or often treated with sulfur dioxide before being sun dried (sulfited dried, or locally known as *Şekerpare*) [9]. They are often treated with sulphur dioxide (also known as “SO<sub>2</sub> fumigation”) as synthetic antioxidant before being sun dried. Fumigation is carried out in rooms of approximately 2.5 × 2.5 × 2.2 m (w:l:h) dimensions; the duration of fumigation is about 10-12 h, and the average consumption of sulphur in this process is 1.6-2 kg sulphur per tonne material [10]. Sulphur dioxide fumigation of apricots provides protection against enzymatic (polyphenol oxidase-catalysed) browning. As high concentrations of sulphite may cause allergic reactions in sensitive individuals, the residual sulphur dioxide levels in dried fruits has been set as 2000 ppm [11]. Another technique to dehydrate the apricot is dehydration under sunlight as a whole or remove the seed and divide to two pieces. Dehydrating of the fresh fruits is provides to remain the fruit without deterioration for a long time.

In this study, dried apricot samples are investigated antioxidant capacity and phytochemical content. The antioxidant activity of the samples is shown by in vitro experiments. Thus, the nutritional value of the dried apricot and its possible effect on human health have been studied.

## 2. Material and Methods

### 2.1. Animals and Chemicals

For experimental studies, two male Wistar albino rats weighing 180-220 g were obtained from Firat University Faculty of Medicine

Experimental Research Center (FUTDAM). Ethics committee report was taken from Firat University Experimental Animal Ethics Board. Chemicals, solvents and standards used in the tests were provided from Sigma-Aldrich.

### 2.2. Herbal Material and Extraction

The samples (SO<sub>2</sub> fumigation, IS; only sun dried, GK) are obtained from the processing of fresh fruits of the species *Prunus armeniaca* found in the Malatya province Inonu University region. IS samples were prepared by drying the fresh fruits under the sunlight after a SO<sub>2</sub> treatment, and GK samples were prepared by drying the fresh fruits under the sunlight directly. Apricot samples were extracted at a ratio of 1:5 (g/mL) with 80% methanol for 4 h. After extracts were centrifuged at 5000 rpm for 5 min and the supernatants were concentrated by drying at under vacuum at 50 °C using a rotary evaporator. Each extract was resuspended in dimethyl sulfoxide (DMSO) to give a stock solution. As a standard, quercetin is prepared in DMSO with a final concentration of 0.04 M.

### 2.3. Tissue Extraction

Two adult albino male rats were killed after anaesthetizing with mild chloroform. 10 g of liver sample decomposed in 100 mL TRIS-EDTA (TE) solution (%3.4 Tris-HCl, %10.5 EDTA, %86.1 H<sub>2</sub>O). After homogenization was centrifuged at 7000 rpm for 10 min. The supernatant portion (ST) is used experimental studies.

### 2.4. Formation of Groups

In order to use them in experimental studies the Control, Fenton reagent (FR), fruit (IS and GK) and standard antioxidant (QE) groups were created. Control group was created 5 mL ST; FR group was created 5 mL ST, 1 mL 40 µM FeCl<sub>2</sub> and 1 mL 60 µM H<sub>2</sub>O<sub>2</sub>; fruit groups and standard antioxidant group included FR plus 1 mL fruit extracts or 100 µL quercetin solution. All of the mixtures were incubated at 37 °C for 24 h. After incubation, 100 µL %4 butylhydroxytoluene (BHT) was added into each

group and vortexed. Lipid peroxidation levels were measured by taking 1 mL of this reaction mixture. Additionally, 100  $\mu$ L was taken from the remaining part and the total protein was measured.

## 2.5. Measurement of the Lipid Peroxidation Level

The lipid peroxidation levels measurements of the samples were determined according to the method of Shimoi et al. [12]. 1 mL of 6% thiobarbituric acid (TBA) was added into the 1 mL of reaction mixture, vortexed, incubated for 45 min at 90 °C. After incubation the samples were cooled to the room temperature, and 3 mL of n-butanol were added and vortexed. The mixture was centrifuged at 5000 rpm for 5 min. Then 1 mL of n-butanol phase was taken into autosampler vials, and intensity of the pink color was measured using a Shimadzu HPLC equipment and RF-10AxL model fluorescence detector. Results were expressed as nmol/mL.

## 2.6. 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  $\lambda$  (excitation) = 515 nm and  $\lambda$  (emission) = 543 nm. Formation of the malonaldehyde (MDA) *in vitro* environment 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<sub>VP</sub>), a fluorescence detector (RF-10<sub>XL</sub>), a column oven (CTO-10AS<sub>VP</sub>), an autosampler (SIL-10AD<sub>VP</sub>), a degasser unit (DGU-14A) and a computer system with class VP software (Shimadzu, Kyoto Japan). Inertsil ODS 3 column (15  $\times$  4.6 mm, 5  $\mu$ m) was used as the HPLC column. The column was eluted isocratically 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 [13].

## 2.7. DPPH Free Radical Scavenging Effect

Measurement of the free radical scavenging effect of the samples was performed according to the method Brand-Williams et al. [14]. The solution of 25 mg/L DPPH in methanol was prepared and 3.9 mL of this solution was mixed with 5, 10, 25 and 50  $\mu$ L concentrations were added onto the stock solutions of IS, GK and QE. The reaction mixture was stored in darkness at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm.

The ability to scavenge DPPH radical was calculated by the following equation:

$$DPPH \text{ radical scavenging activity (\%)} = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

## 2.8. Measurement of Total Protein

Measurement of the total protein amount of the reaction mixture was performed according to the method of Lowry et al. [15]. Bovine serum albumin was used as a standard. Protein amounts of the samples were calculated by creating a calibration curve (Figure 1).

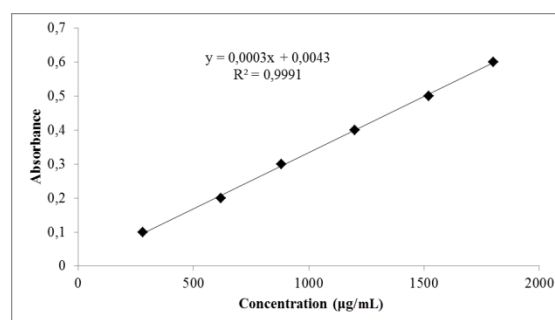


Figure 1. Protein calibration curve.

## 2.9. HPLC Analysis of ADEK Vitamins and Sterol Amount

The plant sterols and  $\alpha$ -tocopherol and were extracted from the lipid extracts by the method of Sanchez-Machado [16] and Lopez-Cervantes et al. [17] with minor modifications. Fruit

sample (1 g) was homogenized with 5 mL hexane and isopropyl alcohol (3:2, v/v). The homogenate was filtered with filter paper. The filtrate was treated with 5 mL of KOH solution (0.5 M in methanol) and immediately vortexed. The tubes were placed in a water bath at 80 °C for 15 min. After cooling in iced water, 1 mL of distilled water and 5 mL of hexane was added and the mixture was rapidly vortexed and then centrifuged for 5 min at 5000 rpm. The supernatant was transferred to another test tube and was kept for drying under nitrogen. The detection was operated using two channels of a diode-array spectrophotometer (326 nm for retinol and 202 nm for  $\alpha$ -tocopherol and phytosterols) [17].

## 2.10. Statistical Analysis

Statistical analysis was performed using SPSS software (version 15.0). The experimental results were reported as mean  $\pm$  SEM (standard error of means). Analysis of variance (ANOVA) and least significant difference (LSD) test were used to compare the experimental groups with the controls. The results were considered statistically significant ( $p < 0.05$ ).

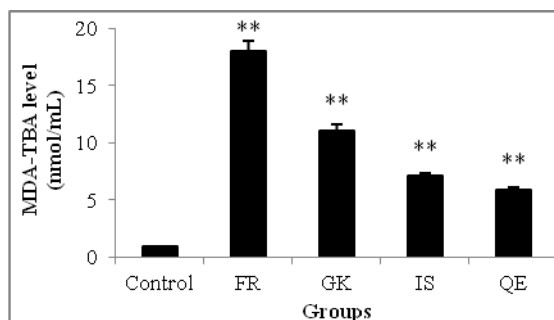
## 3. Results

### 3.1. Lipid Peroxidation Levels

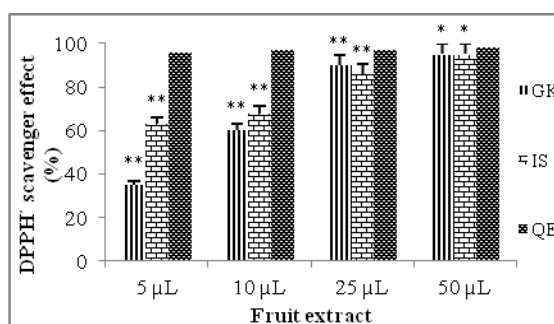
Results of lipid peroxidation levels were shown in the Figure 2. Levels of oxidation in all groups were identified very important rise when compared to the control group ( $p < 0.01$ ). It is observed that the amount of lipid peroxidation in all groups decreased significantly when the FR group was compared with the standard antioxidant and the extract groups ( $p < 0.01$ ).

### 3.2. DPPH Free Radical Scavenging Effect

Analysis results of DPPH free radical scavenging effect of the samples were shown in the Figure 3. Accordingly, antioxidant ability of the samples increased in as dose-dependent. When the apricot extracts were compared with the QE group, a significant difference was found for all concentration.. ( $p < 0.05$ ,  $p < 0.01$ ).



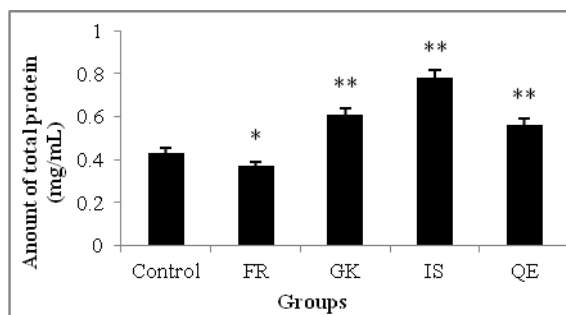
**Figure 2.** The levels of MDA-TBA in the environments of FR and FR with apricot fruit extracts. Data are mean  $\pm$  SEM ( $n=5$ ), FR (Fenton reagent;  $\text{FeCl}_2 + \text{H}_2\text{O}_2$ ), GK (only sun dried), IS ( $\text{SO}_2$  fumigation), QE (Quercetin). \*\*  $p < 0.01$



**Figure 3.** DPPH free radical scavenger effect of apricot fruit extracts (%). Data are mean  $\pm$  SEM ( $n=5$ ), GK (only sun dried), IS ( $\text{SO}_2$  fumigation), QE (Quercetin). \* $p < 0.05$ , \*\* $p < 0.01$

### 3.3. Amount of Total Protein

As shown in Figure 4, compared with the control group, amount of total protein in reactive group decreased ( $p < 0.05$ ), however other groups were determined significantly rise ( $p < 0.01$ ). This result showed that the proteins decreased after oxidation.



**Figure 4.** Levels of total protein of groups FR and FR with apricot fruit extracts. Data are mean  $\pm$  SEM (n=5), FR (Fenton reagent;  $\text{FeCl}_2 + \text{H}_2\text{O}_2$ ), GK (only sun dried), IS ( $\text{SO}_2$  fumigation), QE (Quercetin).

\* $p < 0.05$ , \*\* $p < 0.01$

### 3.4. ADEK Vitamin and Phytosterol Content

Vitamin and phytosterol content of the apricot samples are given in the Table 3.1. As shown in Table 3.1, vitamin D, vitamin K and  $\alpha$ -tocopherol were determined in both apricot samples. There was no difference in terms of the vitamin D amount between the apricot groups ( $p > 0.05$ ), however  $\alpha$ -tocopherol, vitamin K amounts were found high levels in the IS group ( $p < 0.05$ ,  $p < 0.01$ ).

**Table 3.1.** Content of vitamin and phytosterol in apricot samples ( $\mu\text{g/g}$ ).

Vitamin & Phytosterol	GK	IS
Vitamin K	0.023 $\pm$ 0.005	0.037 $\pm$ 0.006*
Vitamin D	0.026 $\pm$ 0.005	0.02 $\pm$ 0.005 <sup>×</sup>
$\alpha$ -tocopherol	0.278 $\pm$ 0.008	0.527 $\pm$ 0.019**
Ergosterol	0.121 $\pm$ 0.007**	0.89 $\pm$ 0.003
Stigmasterol	0.177 $\pm$ 0.012	0.176 $\pm$ 0.009 <sup>×</sup>
$\beta$ -sitosterol	0.019 $\pm$ 0.002	0.048 $\pm$ 0.007**

Data are mean  $\pm$  SEM (n=5), GK (only sun dried), IS ( $\text{SO}_2$  fumigation).

<sup>×</sup>  $p > 0.05$ , \*  $p < 0.05$ , \*\*  $p < 0.01$

According to the results of phytosterol analysis of apricot samples, ergosterol, stigmasterol and  $\beta$ -sitosterol were determined in both samples. Although the amount of

stigmasterol was the same among the groups ( $p > 0.05$ ), the amount of ergosterol was higher in the GK group, and the amount of  $\beta$ -sitosterol was higher in the IS group ( $p < 0.01$ ).

## 4. Discussion

Apricot is a fruit consumed both dried and fresh. Phytochemistry composition and antimicrobial, antimutagenic, cardioprotective and antioxidant effects of the apricot fruit were investigated [7, 18-20]. Egea et al. determined the antioxidant capacity of fresh and industrial processed samples of apricot by measuring their hydroxyl and superoxide radical removal activities. They used BHT, BHA and propyl galatian as a standard and as a result they reported that extracts of apricot had a high radical removal effect [21]. Parlakpınar et al. investigated the effects of feeding with apricots of the rats having myocardial ischemic-reperfusion damage with apricots in their study. At the end of the study, they observed that the ischemic-reperfusion rate decreased significantly in rats fed with apricots compared to the control group. Furthermore, they identified increase in Cu/Zn superoxide dismutase and catalase activities of the hearts of the rats fed with apricots, and a significant decrease in the lipid peroxidation rate [7]. Ozsahin et al. reported that fresh apricot extract inhibited in vitro lipid peroxidation [22].

Reddy et al. studied phenolic compositions and their study was related to antioxidant effects of fresh and dried fruits consumed widely in India, via FRAP and DPPH scavenging activity methods. They reported that the dried apricot extracts showed a significant antioxidant effect [23]. Ozsahin et al. determined antioxidant capacities and phytochemical composition of extracts of apricot fruits. As a result, they showed a preventive effect on the free radical DPPH, depending on the increased extract amounts in all groups. Vitamin D, vitamin K,  $\alpha$ -tocopherol,  $\beta$ -sitosterol, stigmasterol and ergosterol were identified among the vitamins and phytosterols in the samples. Furthermore, resveratrol and flavonoid compounds contained in the samples were also analyzed [18].

Ozturk et al. studied effectiveness of apricot against hepatic steatosis and one of their studies was related to damage induced by carbon tetrachloride (CCl<sub>4</sub>). In the result of the study, it was found that hepatic necrosis and MDA group, total glutathione, catalase, superoxide dismutase and glutathione peroxidase activities increased significantly as a result of oxidative stress in the group induced by CCl<sub>4</sub>. It was identified that the oxidative stress decreased and the histological damage improved in rats fed with apricots. It is concluded that this is the result of antioxidant compounds (β-carotene and vitamins) in apricots [19].

As a result of our study, it is observed that the dried apricot extracts prevent lipid peroxidation against FeCl<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> induced hepatitis oxidation, and preserve the amount of total protein. In addition, their higher activity on DPPH free radical scavenging supports the proposition that apricot fruit is a good antioxidant. It is hypothesized that this antioxidant feature is due to the vitamins, phytosterol and flavonoid compounds identified in the fruit as the result of the analysis. Studies show that these compounds taken dietary, has a significant protective effect against oxidative stress and its damage. In this study, authors assert that the apricot fruits dried various methods can be a good protector against the free radicals that cause great damage in the metabolism.

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