



## Antioxidant Activities, Total Phenolics and Riboflavin Profiles of Turkish Commercial Apricot Nectars

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### Abstract

This study was performed to reveal the total phenolic (TPs) contents, antioxidant activities and riboflavin (RF) (Vitamin B2) contents of eight commercial apricot nectars sold in Turkish markets. The total phenolic (TPs) contents of nectars were analyzed with Folin-Ciocalteu's method. Two comparative antioxidant activities, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing capacities (FRAP) were found for eight nectar samples. A fast capillary electrophoretic method which is combined with laser-induced fluorescence (LIF) detector was applied for the riboflavin analysis. TPs values are ranged between 288-680 mg gallic acid equivalent (GAE)/L, % inhibitions in DPPH method are between 30.58-98.01% and finally FRAP values are between 1.77-5.56 mmol Fe<sup>2+</sup>/L. A high correlation ( $r > 0.893$ ) was observed between two antioxidant test systems. RF contents of apricot nectars are ranged between 20.50-103.37 µg/L. Apricot nectar juices may be considered as a good a supporting dietary source for the daily vitamin B2 intake.

*Keywords:* Apricot nectar, Total phenolics (TPs), DPPH, FRAP, Riboflavin.

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## **Türk Ticari Kayısı Nektarlarının Antioksidan Aktivitesi, Toplam Fenolik Madde ve Riboflavin Profilleri**

### **Özet**

Bu çalışmada; Türk marketlerinde satılan sekiz adet kayısı nektarının toplam fenolik madde (TPs) içerikleri; antioksidan aktiviteleri ve B2 vitamini olarak bilinen Riboflavin içeriğinin tespiti yer almaktadır. Çalışmada kayısı nektarlarının toplam fenolik madde içeriklerinin tayininde Folin-Ciocalteu's yöntemi kullanılmıştır. Antioksidan aktiviteleri için ise hem 2,2-difenil-1-pikrilhidrazil (DPPH) hem de demir indirgeme kapasitesi olarak bilinen FRAP metodları uygulanmıştır. Çalışmanın parçası olan Riboflavin içerik analizlerinde ise hızlı bir yöntem olan Laser İndüklenmiş Floresans (LIF) dedektörü varlığında Kapiler Elektroferez tekniğinden yararlanılmıştır. Çalışılan sekiz adet numunenin toplam fenolik madde içerikleri 288-680 mg gallik asit ekivalent/L aralığında tespit edilirken DPPH yöntemi ile hesaplanan % inhibasyon değerleri 30.58-98.01 aralığında; FRAP değerleri ise 1.77-5.56 mmolFe<sup>2+</sup>/L aralığında bulunmuştur. Kullanılan iki antioksidan tayini çalışmalarının birbiriyle kabul edilir orandaki uyumu hesaplanan korelasyon değeri ile ( $r > 0.893$ ) de desteklenmiştir. Sekiz adet ticari kayısı nektarının Riboflavin içeriklerinin 20.50-103.37 µg/L aralığındad eğiştığı ve budurumda günlük B2 vitamini desteği için kayısı nektarından da faydalanabileceği gözlemlenmiştir.

*AnahtarKelimeler:* Kayısı nektarı, Toplam fenolik (TPs), DPPH, FRAP, Riboflavin.

### **1. Introduction**

Nowadays, fruit juices have a great deal of importance in human diet. Fruit juices are known as a good source of vitamins, minerals and polyphenols, which are the cause of their antioxidant power [1,2]. Commercial juices are either 100% pure fruit juices or nectars which are juices diluted with water and contain different fruit content ranging between 25-40%.

Apricot (*Prunusarmeniaca* L.) is a sweet healthy fruit. Apricot juice is a thick juice and diluted with water and marketed as nectar. Turkey is one of the biggest apricot producer in the world with the 85% of whole apricot production worldwide. The largest amount of harvested apricot in Turkey is used to produce apricot nectar.

Phenolic components [3], minerals [4,5], organic acids, sugars [5-7], antioxidant capacities [5,8-10] and A, C and E vitamin contents [11-14] of apricots were reported so far from different regions of the world. We found only two reported studies on the commercial juices. Carbohydrates, organic acids, amino acids, phenolic compounds and furanic compounds of commercial apricot juices from Italy were identified [15]. Antioxidant capacities and total phenolics (TPs) of four commercial nectar juices from Turkey were investigated [8]. However, we did not find any literature data on vitamin B2 contents of apricots or apricot juices.

Riboflavin (RF), also called as Vitamin B2, is a water-soluble vitamin and essential for human health. Riboflavin participates in many metabolic reactions of carbohydrates, fats, and proteins, and also in blood cell formation. The lack of RF causes some illnesses such as nervous system and dermatological problems. Humans obtain this vitamin from natural foods, but the vitamin B2 content of foods generally remains below the limit of detection of many analytical methods.

Recently, capillary electrophoresis (CE) has taken great attention in food analysis due to its easy method development availability, low sample consumption, fast analysis times, and inexpensive separation columns [16]. In the present study, riboflavin was separated in apricot nectar by CE and detected sensitively by a laser induced fluorescence (LIF) detector coupled with capillary electrophoresis instrument. Total phenolic (TPs) contents and antioxidant activities of eight commercial apricot nectars were determined together with their vitamin B2 contents.

## **2. Materials and Methods**

### **2.1 Chemicals and materials**

Riboflavin, Folin–Ciocalteu reagent, gallic acid, 2,2 diphenyl-1-picrylhydrazyl, 2,4,6-tripyridyl-s-triazine and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  were from Sigma Chemical

Co (Steinheim, Germany). Di-Sodium hydrogen phosphate dehydrate, sodium carbonate anhydrous, sodium acetate trihydrate, and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  were from Merck (Darmstadt, Germany). All solutions were prepared with water purified by an ElgaPurelab Option-7-15 model system (Elga, UK).

Eight commercial apricot nectars (AFN 1-8) were obtained from local markets in Istanbul, Turkey. According to label information of commercial apricot nectars, they have been made from apricot puree with fruit content minimum 40%. One of the commercial apricot juices (AFN8) is called apricot flavored mixed fruit juice made up by concentrated fruit juices, and fruit content given as 13% apricot, 6% orange and 1% apple.

## **2.2 Sample Preparation**

All apricot nectars were centrifuged at 6000 rpm for 5 minute. After filtering through a  $0.45\mu\text{m}$  membrane filter, the samples were ready for all analysis.

## **2.3 Capillary Electrophoretic determination of Riboflavin (RF)**

Separations were performed with an Agilent1600 capillary electrophoresis system (Waldbronn, Germany) equipped with a ZETALIF 2000 LIF detector (Picometrics, Montlaur, France). RF was detected with an excitation at 488 nm and emission at 520 nm by an Ar-ion laser. The data processing was carried out with the Agilent ChemStation software. The separation was performed at 25 kV. The temperature was set at 25 °C. Injections were made at 50 mbar for 6 s. The fused-silica capillary used for separation experiments was 50  $\mu\text{m}$  id and was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 67 cm and the length to the detector was 50 cm. The new fused-silica capillary was conditioned prior to use by rinsing with 1 M NaOH for 30 min and with water for 10 min. The capillary was flushed successively by 0.1 M NaOH for 2 min, water for 2 min, and buffer for 5 min at the beginning of every working day and between runs.

The analysis method which was lately developed for riboflavin determination in honey [17] and saffron samples [18] by our group was slightly modified for the analysis of nectars in this study.

It was detected that the fluorescence intensity of RF depends on the pH of the separation medium and the fluorescence intensity of riboflavin is in maximum at pH around 9.5. Since the pKa value of RF is 9.69, at these pHs riboflavin gains negative charge and migrate in the applied electrical field. Phosphate and borate electrolytes were tried in the preliminary experiments and phosphate gave better results in terms of peak height and peak symmetry of the RF standard solution. The phosphate concentration was changed between 15 and 75 mmol/L and no significant change was observed in peak shapes. Finally, 30 mmol/L phosphate at pH 9.9 was selected as the optimal separation medium.

#### **2.4 Determination of Total Phenolics (TPs)**

Total phenolics (TPs) of apricot nectars were determined by using Folin-Ciocalteu method [19]. Three hundred  $\mu$ L of fruit nectars were mixed with 1.5 mL of Folin-Ciocalteu's reagent (1:10 diluted with water) and 1.2 mL of sodium carbonate solution (7.5% w/v). The mixture was allowed to stand for 10 minutes at room temperature until a stable color was obtained. The absorbance values of 1/10 fold diluted samples were measured by a Shimadzu UV-1800 spectrophotometer at 760 nm. Results were expressed as gallic acid equivalents (GAE) in mg/L. The calibration equation for gallic acid was  $y = 49.582x - 0.0185$  ( $R^2 = 0.995$ ).

#### **2.5 Determination of Antioxidant Activities**

The antioxidant activities of apricot nectars were determined by two methods. The first method is based on the evaluation of the free-radical scavenging capacity. In this method, the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical was used to measure the antioxidant activity of nectars [20]. In this method 20 mg/L DPPH stock solution was prepared in methanol. 50  $\mu$ L of fruit nectars were mixed with 1950  $\mu$ L DPPH stock solution. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was measured at 517 nm. Radical scavenging activity was expressed as the

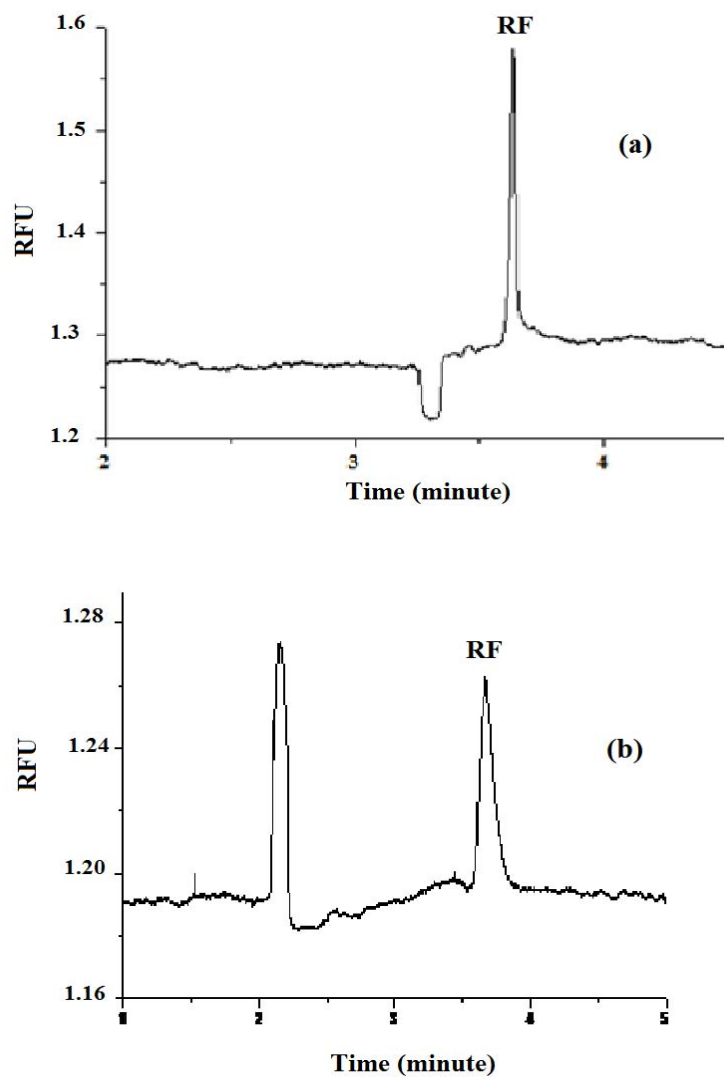
inhibition percentage. The second method applied here is based on the measurement of the ferric-reducing capacity of nectars. The ferric-reducing antioxidant powers (FRAP) of fruit nectars were determined, following the method of Benzie and Strain [21]. FRAP reagent was prepared containing 1:1:10 ratio of 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mmol/L HCl, 20 mmol/L FeCl<sub>3</sub> and 0.3 mol/L acetate buffer at pH 3.6, and warmed to 37°C for 10 min prior to use. The mixture which containing 100 µL sample, 100 µL deionized water and 1.8 mL FRAP reagent incubated at 37°C for 10 min. The absorbance of the 1/10 fold diluted mixture was measured by a Shimadzu UV-1800 spectrophotometer at 593 nm. Results were expressed as mmol Fe<sup>+2</sup>/L. The calibration equation for FeSO<sub>4</sub>.7H<sub>2</sub>O was  $y = 20.044x - 0.0373$  ( $R^2 = 0.999$ ).

### **3. Results**

#### **3.1 Riboflavin (RF)**

Calibration curve of RF was linear between 0.01-5 µM concentration ranges. The calibration equation was calculated as  $y = 0.9544x - 0.0375$  ( $R^2 = 0.999$ ). The limit of detection (LOD) was calculated as 3 times of the average noise taken for three different baseline areas and found as 2.86 nM. The limit of quantification (LOQ) was given as ten times the average noise as 9.53 nM. For the precision of the method, the riboflavin standard solution was injected 5 times in one day. For the day-to-day reproducibility, the same solution was injected five times in three different non-consecutive days. In the same day precision of the corrected peak areas (%RSD) was 2.48%. Between days, precision value was 4.58%.

After centrifuging and filtrating processes, nectars were injected three times and riboflavin peak was detected in less than 4 min. Figure 1(a) and Figure 1(b) show both the electropherogram of 0.3 µM of Riboflavin (RF) standart solution and also one commercial apricot fruit nectar (AFN 3).



**Figure 1.** Electropherogram of (a) 0.3  $\mu\text{M}$  of Riboflavin (RF) standart solution; (b) commercial apricot fruit nectar (AFN 3). Conditions: Capillary: 50  $\mu\text{m}$ i.d. and 50x67 cm; Injection: 50 mbar 6s; Voltage: 25 kV; Separation electrolyte: 30 mM phosphate at pH: 9.9

The riboflavin contents of nectar samples are given in Table1. As demonstrated in Table 1, RF contents of commercial apricot nectars are ranged between 36.74-103.4  $\mu\text{g/L}$ , whereas riboflavin content of apricot containing mix juice (AFN 8) is 20.50  $\mu\text{g/L}$ . Thereby, we can say that the amount of riboflavin in nectars increases with the increase

in the apricot contents. Beef and liver contain higher amount of vitamin B2 compared to foods in human diet. Green vegetables and some plant teas are the other sources of vitamin B2. Using CE-LIF method, riboflavin contents were reported in some vegetables between 0.34 and 1.67  $\mu\text{g/g}$  [22], and in green tea as 2.8-5.4  $\mu\text{g/g}$  [23]. Comparatively, apricot nectar is an alternative source of vitamin B2 in diet.

**Table 1.** RF content of apricot fruit nectars

<b>Apricot Nectars</b>	<b>Riboflavin (<math>\mu\text{g/L} \pm \text{SD}</math>)</b>
<b>AFN 1</b>	98.93 $\pm$ 1.90
<b>AFN 2</b>	46.05 $\pm$ 2.99
<b>AFN 3</b>	67.66 $\pm$ 1.28
<b>AFN 4</b>	74.50 $\pm$ 1.46
<b>AFN 5</b>	103.4 $\pm$ 7.8
<b>AFN 6</b>	41.31 $\pm$ 3.58
<b>AFN 7</b>	36.74 $\pm$ 2.20
<b>AFN 8</b>	20.50 $\pm$ 0.30

### 3.2 Total Phenolic (TPs) and Antioxidant Activities

TPs (mg GAE/L) and antioxidant activities of nectar samples in terms of DPPH (% inhibition) and FRAP values (mmol  $\text{Fe}^{2+}$ / L) are given in Table 2.

**Table 2.** TPs, DPPH and FRAP values of the samples

<b>Apricot Nectars</b>	<b>(TPs) (mg GAE/L <math>\pm</math> SD)</b>	<b>DPPH (% inhibition <math>\pm</math> SD)</b>	<b>FRAP (mmol<math>\text{Fe}^{2+}</math>/ L <math>\pm</math> SD)</b>
<b>AFN 1</b>	680 $\pm$ 33	98.01 $\pm$ 0.45	5.56 $\pm$ 0.06
<b>AFN 2</b>	573 $\pm$ 11	93.68 $\pm$ 0.90	4.48 $\pm$ 0.36
<b>AFN 3</b>	386 $\pm$ 14	63.27 $\pm$ 3.82	2.65 $\pm$ 0.00
<b>AFN 4</b>	425 $\pm$ 10	84.81 $\pm$ 1.55	4.30 $\pm$ 0.03
<b>AFN 5</b>	642 $\pm$ 15	94.17 $\pm$ 0.68	5.48 $\pm$ 0.01
<b>AFN 6</b>	377 $\pm$ 14	58.67 $\pm$ 1.74	2.24 $\pm$ 0.01
<b>AFN 7</b>	525 $\pm$ 24	92.89 $\pm$ 1.05	5.22 $\pm$ 0.02
<b>AFN 8</b>	288 $\pm$ 11	30.58 $\pm$ 1.61	1.77 $\pm$ 0.04

According to antioxidant results, the correlation value between two methods (DPPH and FRAP) is greatly satisfied ( $r > 0.893$ ).



In the literature there is only one FRAP data for apricot nectar which is published in 2003 by Tosun and Ustun from Turkey [24]. They reported the FRAP value as between 5.2-6.0 mmol Fe<sup>2+</sup> /L for apricot nectars which are greatly compatible with our results. Similarly, Karav and Eksi reported in 2012 the TP values between 501.9-625.4 mg GAE/L for commercial apricot nectars in Turkey [8] which are close to our results.

#### **4. Conclusions**

Antioxidant activities, total phenolic (TPs) contents and RF (Vitamin B2) profiles of eight Turkish commercial apricot nectar juices are identified, considering the consumer health and the knowledge for fruit juice industry. Commercial nectar juices were found are good antioxidant and vitamin B2 sources in daily diet.

#### **Acknowledgment**

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