Journal of Food and Nutrition Research, 2015, Vol. 3, No. 10, 624-628
Available online at http://pubs.sciepub.com/jfnr/3/10/1

DOI:10.12691/jfnr-3-10-1

Apple Peel is a Promising Source of Natural Bioactive Compounds That Promote Human Health

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Abstract Antioxidant capacities (AC) and total phenolic content (TPC) of sixteen apple varieties obtained from different regions of Turkey and six commercial apple juices were examined. Trolox Equivalent Antioxidant Capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assays were used for AC measurement while Folin-Ciocalteu method was applied for TPC measurements. 'Ankara Agustos' cultivar showed the highest antioxidant capacity (7.60 µmol TE/g FW) followed by 'Bursa Gloster' (3.10 µmol TE/g FW), and 'Antalya Red Chief' (2.90 µmol TE/g FW), whereas 'Antalya Granny Smith' exhibited the lowest antioxidant capacity (1.50 µmol TE/g FW). AC's of commercial apple juices were found to be 2.8-fold less than the average of naturally produced juices with range of 0.38-2.56 µmol TE/g FW. Total antioxidant capacity changes at 4°C over time were also monitored for three varieties. A significant decrease in total antioxidant capacity after five weeks was observed. Moreover, the high correlation between AC and TPC of naturally produced apple juices (R^2>0.97) indicated that phenolic compounds are a major contributor to the antioxidant activity of apple fruit.

Keywords: *pomegranate, antioxidant capacity, phenolic content*

Cite This Article: Sercan Karav, Ardic O. Arikal, and Aziz Eksi, "Apple Peel is a Promising Source of Natural Bioactive Compounds That Promote Human Health." *Journal of Food and Nutrition Research*, vol. 3, no. 10 (2015): 624-628. doi: 10.12691/jfnr-3-10-1.

1. Introduction

Free radicals are naturally formed as a part of normal metabolic processes. These compounds constitute a class of chemical species that are known to be extremely unstable, reactive and toxic [1,2,3]. Extensive research have demonstrated that the overproduction of reactive oxygen species (ROS) including hydroxyl radicals, superoxide anions, and hydrogen peroxide can cause DNA damage, protein oxidation and lipid peroxidation in living cells [4,5,6]. In addition, excessive ROS production may result in several clinical conditions such as cardiovascular diseases, diabetes, liver injury, cancer and aging [7,8,9]. However, the innate defense system of human body may not be sufficient to neutralize severe or continued oxidation caused by ROS. Therefore, input of exogenous antioxidants is critically important to maintain the balance of the oxidative stress in human body [10].

Antioxidant activity is described as the ability to reduce free radical formation and scavenge ROS [11]. Research on characterization of exogeneous antioxidants show that antioxidant effect of plants is mainly contributed by their phenolic components such as flavonoids, phenolic acids and phenolic diterpens that have the ability to scavenge free radicals and donate hydrogen atoms or electrons [12]. Plant polyphenols have also potential benefits on human health due to their anti-inflammatory, antiviral, antimicrobial, and antioxidant activity [13,14].

Consumption of fruits and vegetables plays an important role in prevention of many chronic diseases because of their high antioxidant activity [15,16,17]. Although many fruits are known sources of antioxidants their antioxidant activity varies considerably even within the same species due to differences in genotype, growing temperature, growing season, maturity at harvest, environmental stresses and post-harvest treatments [18-23]. Apple (*Malus domestica* Borkh.), a member of the family *Rosaceae*, is one of the most consumed fruits in many regions across the world. Apple fruit has been identified as an excellent source of carbohydrates, minerals, dietary fibers and antioxidant phenolics [15,24,25]. Apple represents a major source of dietary antioxidants among other fruits and vegetables owing to its widespread consumption and availability in the market during every season. Apple peel is also valued for its health promoting functions due to its high dietary fiber and phenolic content [24].

The objective of this study was to determine the total antioxidant capacity of naturally produced and commercial apple juices and find the correlation with total phenolic content. Different methods including ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC), and 2,2'-diphenil-1-picrylhydrazyl (DPPH) radical-scavenging activity [26,27,28,29,30] were used to asses antioxidant or antiradical activity of the apples. Despite the wide variety of antioxidant capacity measurement methods a high correlation between their results have been reported [31,32,33,34]. Therefore, we

also employed aforementioned methods and analyzed the consistency of their results. In addition, we address the differences in antioxidant capacities of apple peel and flesh. Moreover, total antioxidant changes over time at 4°C were screened for three varieties to elucidate the stability of antioxidant activity under refrigeration conditions.

2. Materials and Methods

In this study, natural apple juices produced from sixteen different apple cultivars collected from different regions of Turkey and six commercial apple juices obtained from popular fruit juice companies were examined. The varieties and growth regions of samples are given in Table 1. Fruit samples were processed to fruit juices in laboratory scale and stored at -25 °C until analysis.

Table 1. Regional Distribution and Descriptive Values of Apple Samples

Growing region	Variety	Soluble solid content(°Brix)	Titratable acidity (%)
Ankara	Golden Delicious	13.3±0.2	0.33±0.01
Ankara	Agustos	17.4±0.3	0.92±0.02
Amasya	Amasya	8.3±0.1	0.23±0.01
Bursa	Fuji	9.5±0.2	0.45±0.01
Bursa	Gloster	12.2±0.3	0.58±0.02
Manisa	Granny Smith	9.0±0.2	0.85±0.01
Ankara	Arapkızı	11.7±0.2	0.37±0.03
Corum	Starking	11.6±0.2	0.34±0.01
Corum	Breaburn	9.2±0.1	0.64±0.02
Manisa	Golden Delicious	11.5±0.1	0.58±0.02
Antalya	Granny Smith	11.5±0.2	0.44±0.01
Antalya	Red Chief	12.5±0.3	0.79±0.01
Mugla	Fuji	13.4±0.2	0.59±0.01
Izmir	Golden Delicious	12.9±0.2	0.54±0.01
Izmir	Fuji	16.9±0.2	0.61±0.01
Izmir	Breaburn	13.8±0.2	0.65±0.02

*Values shown represent means ±S.D of triplicate measurements.

2.1. Determination of the Original Characteris of the Samples

Apart from the antioxidant and total phenolic content analyses, soluble solid content (Brix) and titratable acidity of samples were determined in order to describe the original characteristics of the fruits. Soluble solid contents of the fruits were determined with Bausch & Lomb (USA) refractometer at 20°C and expressed as °Brix (Anonymous 1991). 2 mL aliquot of sample was diluted with 20 mL distilled water, and the total titratable acidity was determined by titrating the sample with 0.1 M NaOH until the pH reached 8.1 and expressed as grams of citric acid per liter (Anonymous 1996).

2.2. TRAC and FRAP Assays to Monitor Scavenging Activity

The TEAC assay was carried out according to the method of Re *et al* [35]. First, to produce the radical cation $ABTS^{\bullet+}$, 7 mmol/L ABTS salt and 2.45 mmol/L potassium persulfate were mixed in a volume ratio of 1:1. Subsequently, the reaction mixture was allowed to stand in the dark for 16 h at room temperature (25°C) and was used within two days of preparation. The $ABTS^{\bullet+}$ radical solution was diluted with ethanol to an absorbance of 0.7±0.05 at 734 nm. All samples were diluted approximately to provide 20–80% inhibition of the blank absorbance. 100 microliters of the diluted sample was mixed with 3.8 mL $ABTS^{\bullet+}$ working solution, and the reaction mixture was left at room temperature to react for 6 min, then the absorbance at 734 nm was measured using the ultraviolet spectrophotometer. Trolox solution was used as a reference standard, and the results were expressed as µmoles of Trolox per gram of sample fresh weight (µmol TE/g FW). The DPPH assay was carried out according to the method of Brand-Williams *et al.* ([28] with some modifications. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and was stored at -20°C. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using the spectrophotometer. Samples (150 mL) were allowed to react with 2850 mL of the DPPH solution for 24 h in the dark. Then the absorbance was measured at 515 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in µmoles of Trolox per gram of sample (µmol TE/g FW).

2.3. Total Phenolic Assay

Total phenolic content was determined with the Folin-Ciocalteu reagent according to the procedure described by Rentschler and Tanner [36]. Briefly, 0.50 mL of the diluted sample was reacted with 2.5 mL of 0.2 mol L^{-1} Folin-Ciocalteu reagent for 4 min, then 2 mL saturated sodium carbonate solution (about 75 g L^{-1}) was added into the reaction mixture. The absorbance at 760 nm was measured following the incubation at room temperature for 2 h. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent per grams fresh weight of the sample (mg GAE/g FW).

2.4. Statistical Analysis

Analyses were performed in 3 parallel trials and the descriptive statistics were studied employing Minitab 16.0. One-way ANOVA was performed and Tukey test was used for multiple comparisons of the groups at significance level of 0.05.

3. Results and Discussion

The soluble solid content (°Brix) of apple varieties and commercial apple juices were found within the range of 8.3-17.4 and 13.3-16.8, respectively, while % titratable acidity (TA) of the samples were 0.23-0.92 and 0.76-0.92, respectively. These results are important for the understanding of the basic characteristics of different apple varieties since different soluble solid content and TA values might lead to disparities in total antioxidant capacity. The total antioxidant capacity and total phenolic contents of apple flesh and peels were compared as shown in Table 2 and Table 3. The total antioxidant values of

apple flesh were within the range of 1.50-7.60 and 0.50-2.52 µmol TE/g, while antioxidant capacities of the peels were between 2.89-14.60 and 1.06-4.93 µmol TE/g fw for TEAC and DPPH assays, respectively. These results showed that there is a high correlation between (R^2=0.92 (flesh) and 0.89 (peel)) TEAC and DPPH (Figure 1), in agreement with other studies [37,38,39,40]. The total phenolic contents of flesh were found as 0.14-0.28 mg CAE/g FW, while the values for peels were between 0.29-0.69 mg GAE/g FW. It was shown that there is a significant correlation between total antioxidant capacity and total phenolic contents of both apple flesh and peels (R^2= 0.97 and 0.89, respectively) (Figure 2). These results suggest that the phenolic compounds are major contributors for the antioxidant capacity of apple. Moreover, the average of the total antioxidant capacity and total phenolic content of apple peels is over 2.9 fold more than the average of apple flesh values. Markedly higher antioxidant and phenolic content of apple peel points to a potential benefit of including it during the fruit juice processes to increase the value of the product.

Table 2. Total Antioxidant (TA) Capacity and Total Phenolic (TP) content of Flesh of Apple Varieties

Region	Variety	TA TEAC (µmol TE/g FW)	TA DPPH (µmol TE/g FW)	TP (mg GAE/g FW)
Ankara	Golden Delicious	2,56^{a}	0.84^{a}	0,17^{a}
Ankara	Agustos	7,60^{b}	2.52^{b}	0,28^{b}
Amasya	Amasya	2,83^{c}	0.93^{c}	0,18^{a}
Bursa	Fuji	2,18^{d}	0.65^{d}	0,15^{c}
Bursa	Gloster	3,10^{e}	0.89^{a}	0,19^{a}
Manisa	Granny Smith	1,67^{f}	0.5^{e}	0,14^{c}
Ankara	Arapkızı	2,62^{a}	0.53^{e}	0,17^{a}
Corum	Starking	4,50^{g}	1.18^{f}	0,22^{d}
Corum	Breabun	2,25^{d}	0.79^{g}	0,16^{a}
Manisa	Golden Delicios	2,70^{h}	0.56^{e}	0,17^{a}
Antalya	Granny Smith	1,50^{i}	0.50^{e}	0,14^{c}
Antalya	Red Chief	2.90^{c}	1.02^{h}	0,18^{a}
Mugla	Fuji	2.55^{a}	0.76^{g}	0,16^{a}
Izmir	Golden Delicios	2.46^{j}	0.69^{d}	0,16^{a}
Izmir	Fuji	2.25^{d}	0.64^{d}	0,16^{a}
Izmir	Breabun	2.80^{c}	0.81^{g}	0,18^{a}

* The values (a-j) with the same letter within a column shows no significant difference (p=0.05).

Table 3. Total Antioxidant (TA) Capacity and Total Phenolic (TP) Contents of Peel of Apple Varieties

Region	Variety	TA TEAC (µmol TE/g FW)	TA DPPH (µmol TE/g FW)	TP(mg GAE/g FW)
Ankara	Golden Delicious	9.60^{a}	3.35^{a}	0,45^{a}
Ankara	Agustos	14.60^{b}	4.93^{b}	0,69^{b}
Amasya	Amasya	10.20^{c}	3.45^{c}	0,45^{a}
Bursa	Fuji	7.50^{d}	2.89^{d}	0,39^{c}
Bursa	Gloster	11.80^{e}	4.05^{e}	0,59^{d}
Manisa	Granny Smith	2.89^{f}	1.06^{f}	0,29^{e}
Ankara	Arapkızı	9.90^{a}	3.35^{a}	0,47^{f}
Corum	Starking	11.20^{g}	3.99^{g}	0,60^{d}
Corum	Breabun	7.65^{d}	2.19^{h}	0,45^{a}
Manisa	Golden Delicious	10.80^{h}	3.65^{h}	0,48^{a}
Antalya	Granny Smith	3.65^{i}	1.89^{i}	0,27^{e}
Antalya	Red Chief	7.65^{d}	1.79^{j}	0,46^{f}
Mugla	Fuji	8.89^{j}	3.06^{k}	0,47^{f}
Izmir	Golden Delicios	8.70^{j}	3.02^{k}	0,45^{a}
Izmir	Fuji	4.45^{k}	1.12^{f}	0,34^{g}
Izmir	Breabun	6.54^{l}	2.65^{l}	0,40^{c}

* The values (a-l) within a column with the same letter are not significantly different (p = 0.05).

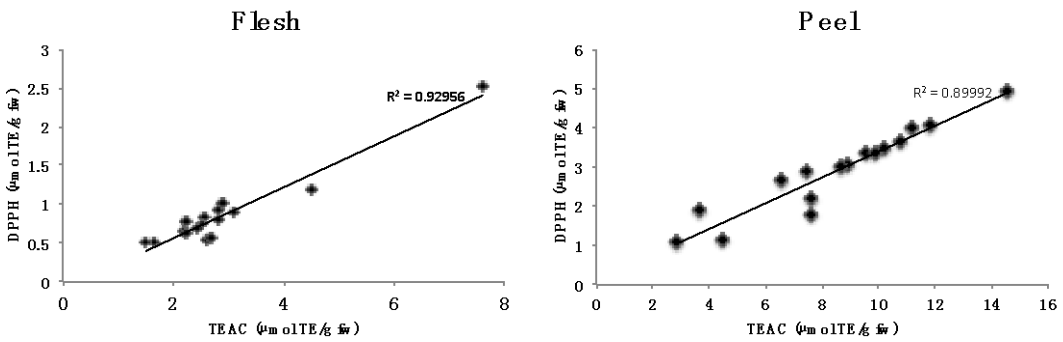

Figure 1. The correlation of TEAC and DPPH methods on total antioxidant capacities of apple flesh and peel

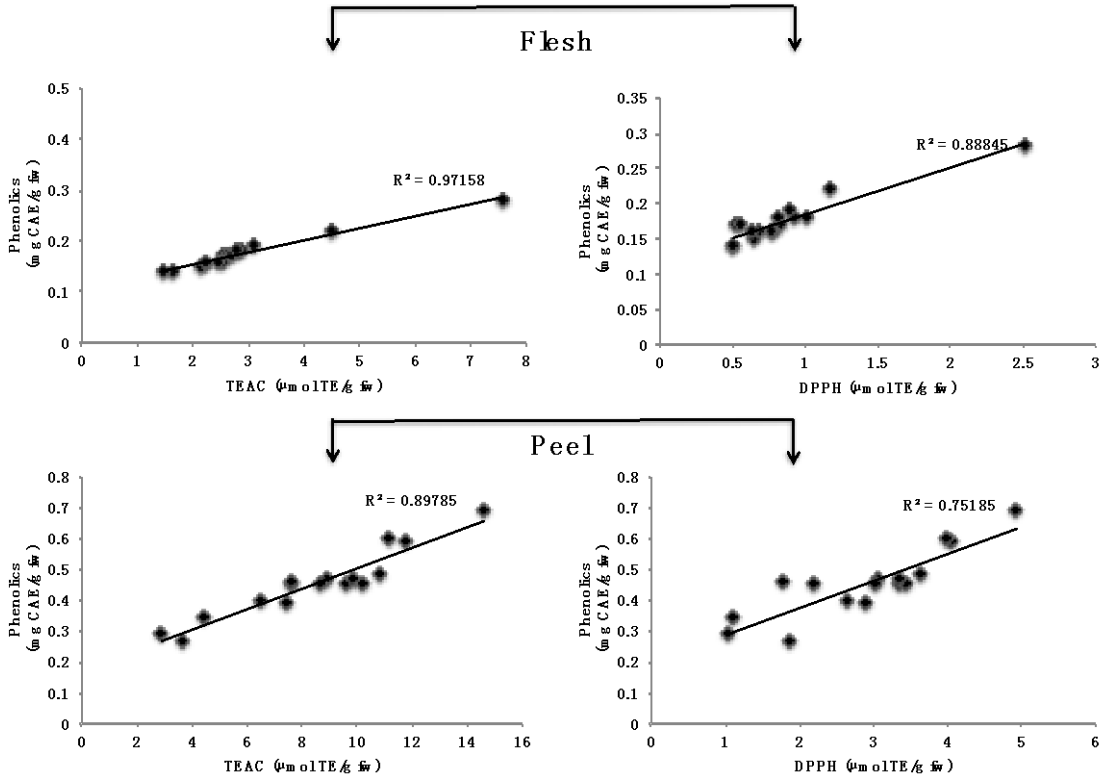

Figure 2. The correlation of total antioxidant capacity (TEAC and DPPH) and total phenolic content of apple flesh and peel samples

Six 100% commercial apple juice collected from famous companies in Turkey were analyzed to determine the total antioxidant activity and phenolic content (Table 4). The antioxidant capacity of the samples varied between 0.38-2.56 µmol TE/g while the total phenolic content of the juices were between 0.55-1.48 mg GAE/g fw. The significant difference between the samples is a likely result of the apple varieties used for the juice production. As shown above, different apple varieties might result in different total antioxidant capacities after processing. Moreover, different processing techniques might change the total antioxidant content of the juices. For example, processing the apples without peel removal might increase the total antioxidant capacity.

Table 4. Total Antioxidant (TA) and total phenolic (TP) Contents of Commercial Apple Juices

Commercial apple juice code	Soluble solid content(°Brix)	Titratable acidity (%)	TA TEAC (µmol TE/g FW)	TP (mg GAE/g FW)
A	13.3±0.2	0.92±0.04	0.38±0.02	0.55±0.04
B	15.4±0.1	0.89±0.07	0.70±0.03	0.90±0.02
C	16.2±0.4	0.85±0.03	1.10±0.03	0.95±0.01
D	15.6±0.3	0.79±0.04	2.56±0.11	1.48±0.04
E	15.2±0.2	0.79±0.06	0.95±0.04	0.74±0.04
F	16.8±0.3	0.76±0.02	0.87±0.02	0.60±0.04

* TA represents percent titratable acidity. Values shown represent means ±S.D of triplicate measurements.

To determine the total antioxidant changes of apple juices kept in refrigerator temperature (4°C), three samples were used that have high, medium and low antioxidant capacity. The total antioxidant capacity of 'Golden Delicious', 'Granny Smith' and 'Agustos' varieties were screened at 4°C for two months (Figure 3). It was shown that first 4 weeks, total antioxidant capacity of samples were stable for all three samples. However, total antioxidant capacity of 'Agustos' variety decreased from 6.66 to 3.22 µmol TE/g in week 5, while the antioxidant capacity of both 'Golden Delicious' and 'Granny Smith' dramatically decreased from 2.55 to 1.6 µmol TE/g and 1.5 to 0.7 µmol TE/g, respectively. These results show that there is no significant decrease in antioxidant capacity of apple juices. The decrease after during week 5 might be the result of the degradation of phenolic compounds which are the main contributor of total antioxidant activity.

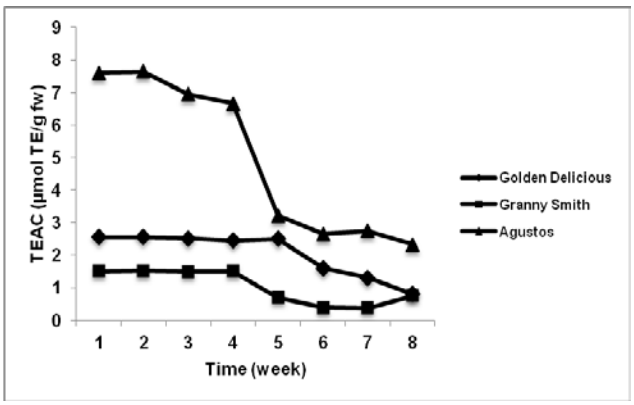

Figure 3. The changes in total antioxidant content in three different varieties of apples during storage at 4°C

4. Conclusions

In this study, we investigated the total antioxidant capacity and phenolic content of sixteen apple varieties grown in Turkey and six commercial juices. We showed that there is a significant difference in total antioxidant activity between apple varieties. The high correlation between total antioxidant capacity and phenolic content showed that phenolic compounds are the major contributor for the antioxidant capacity. Moreover, we observed that apple peel is rich in phenolics which increases the total antioxidant capacity. It was also shown that commercial apple juices have significantly different in antioxidant capacities. We found that the storage of apple juices at 4°C did not affect the antioxidant capacity for the first four weeks, while there was a dramatic decrease after 5 weeks for all samples.

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