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Antioxidant Activity of Selected Fruits and Vegetables Grown in Turkey

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Abstract: Antioxidant activities of different fruits (apple, quince, grape, pear and pomegranate) and vegetables (potato, onion, spring onion, red radish and red cabbage) were determined. In addition, total phenolic and flavonoid contents of those samples were assessed. Among fruits, pomegranate had the highest (62.7%) antioxidant activity, followed by quince (60.4%), grape (26.6%), apple (25.7%) and pear (13.7%). The antioxidant activity of vegetables ranged from 40.8% (red cabbage) to 12.5% (onion). Total phenolic and flavonoid contents in fruits varied from 326 to 4306 mg of catechin kg⁻¹ and from 282 to 2115 mg of catechin kg⁻¹, respectively. Those in vegetables ranged between 536 and 2166 mg of catechin kg⁻¹ and between 153 and 842 mg of catechin kg⁻¹, respectively. A high and significant correlation between antioxidant activity and total phenolic content was determined in fruits ($r^2 = 0.9307$, $P < 0.01$) and vegetables ($r^2 = 0.9361$, $P < 0.05$). However, flavonoid content was not significantly correlated with antioxidant activity in vegetables, while it was significantly related in fruits ($r^2 = 0.8316$, $P < 0.01$). It was observed that total phenolic content is the major contributor to the antioxidant activity of fruits and vegetables.

Key Words: Antioxidant activity, total phenolics, total flavonoids, fruits, vegetables

Türkiye'de Yetişen Bazı Meyve ve Sebzelerin Antioksidan Aktivitesi

Özet: Farklı meyve (elma, ayva, üzüm, armut ve nar) ve sebzelerin (patates, soğan, taze soğan, kırmızı turp ve kırmızı lahana) antioksidan aktiviteleri belirlenmiştir. Ayrıca, toplam fenolik ve flavonoid içerikleri de değerlendirilmiştir. Meyveler arasında nar (% 62.7) en yüksek antioksidan aktiviteye sahip olup bunu ayva (% 60.4), üzüm (% 26.6), elma (% 25.7) ve armut (% 13.7) izlemektedir. Sebzelerin antioksidan aktivitesi % 40.8 (kırmızı lahana) ile % 12.5 (soğan) arasında değişmektedir. Meyvelerin toplam fenolik ve flavonoid içerikleri sırasıyla 326-4306 mg kateşin kg⁻¹ ve 282-2115 mg kateşin kg⁻¹ arasındadır. Sebzelerde ise bu değerler sırasıyla 536-2166 mg kateşin kg⁻¹ ve 153-842 mg kateşin kg⁻¹ olarak belirlenmiştir. Meyve ($r^2 = 0.9307$, $P < 0.01$) ve sebzelerde ($r^2 = 0.9361$, $P < 0.05$) toplam fenolik içerikleri ve antioksidan aktiviteleri arasında yüksek ve önemli bir korelasyon belirlenmiştir. Bununla birlikte, sebzelerin antioksidan aktiviteleri ile flavonoid içerikleri arasındaki ilişki önemsiz iken meyvelerde bu ilişki önemlidir ($r^2 = 0.8316$, $P < 0.01$). Toplam fenolik içeriğinin meyve ve sebzelerin antioksidan aktivitelerine önemli katkıda bulunduğu gözlenmiştir.

Anahtar Sözcükler: Antioksidan aktivite, toplam fenolik, toplam flavonoid, meyve, sebze

Introduction

Interest in the role of antioxidants in human health has prompted research in the fields of food science and horticulture to assess fruit and vegetable antioxidants (Kalt et al., 1999). The majority of the antioxidant capacity of a fruit or vegetable may be from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins rather than from vitamins C, E or β -carotene (Wang et al., 1996; Kähkönen et al.,

1999). Many of these phytochemicals may help to protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002). Fruit and vegetable antioxidants play an important role in reducing the risk of degenerative diseases such as cardiovascular disease, various cancers and neurological diseases (Kalt et al., 1999).

There are approximately 5000 known plant phenolics and model studies have demonstrated that many of them

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have antioxidant activity (Robards et al., 1999). The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans et al., 1995). Their antioxidant activity is generally based on the number and location of hydroxyl groups present as well as the presence of a 2-3 double bond and 4-oxofunction (Rice-Evans and Miller, 1998). The flavonoids, a large family of low molecular weight polyphenolic compounds, include the flavones, flavonols, flavonones, isoflavones, flavan-3-ols and anthocyanins (Stewart et al., 2000). Although flavonoids are generally considered non-nutritive agents, interest in these substances has risen because of their possible effects on human health (Hertog et al., 1992). In addition to their antioxidant activities, flavonoids inhibit enzymes such as prostaglandin synthase, lipoxygenase and cyclooxygenase, closely related to tumorigenesis, and may induce detoxifying enzymes such as glutathione S-transferase (Lee et al., 1995). Many kinds of flavonoid have been reported in fruits and vegetables and their types and contents vary with cultivar and maturation (Hertog et al., 1992).

All of these aspects explain the increasing interest in fruit phenolics that has been manifested in the past few years. In this context, a large number of plant sources including many fruits and vegetables have been explored for their antioxidant potential. Therefore, the main objective of this study was to determine the antioxidant activity of different fruits and vegetables grown in Turkey. Another aim was to evaluate whether total phenolic and flavonoid contents of samples are correlated with antioxidant activity.

Materials and Methods

Materials

All the apple (Amasya, Arap Kızı, Cooper, Gloster, Golden Delicious, Granny Smith, Rome Beauty and Starking) and quince (Çiçek Dağı, Çubuk, Çukur Göbeği, Ekmek, Eşme, Kalecik, Kirli and Yerköy) varieties and 1 variety of grape (Müşküle) were harvested from the Department of Horticulture's trial areas (Department of Horticulture, University of Ankara). The other variety of grape (Seedless Sultana), and all varieties of pear (Ankara, Deveci and Santa Maria), pomegranate and vegetables (spring onion, onion, potato, red radish and

red cabbage) were purchased fresh from local markets in Ankara in winter. All fruits and vegetables were washed and grated before extraction. Only pomegranate, onion and potato were peeled before the extraction process, and pomegranate was sectioned and the seed coats were removed.

Extraction

The phenolics from samples were isolated by a modified version of the method described by Shahidi et al. (2001). One gram of sample was extracted 3 times with 10 ml of 70% (v/v) aqueous methanol (Merck, Darmstadt, Germany) at room temperature using an Ultra Turrax T25 homogenizer (IKA Werke Labortechnik, Staufen, Germany) at 11,000 rpm for 1 min. The slurry was centrifuged at 4000 rpm for 15 min. Supernatants were collected and combined in a rotary flask and then evaporated to dryness at 45 °C under vacuum with a Rotavapor R-3000 rotary evaporator (Buchi, Switzerland). The extracted phenolics were dissolved in 25 ml of methanol and then filtered using filter paper. Methanolic solutions of phenolics were stored at -26 °C until analysis. Extractions and all analysis given below in detail were performed in 2 replicates.

Determination of antioxidant activity

Antioxidant activity of extracts and of some common antioxidants (α -tocopherol from Supelco Park, Bellefonte, PA, USA; BHA and BHT from Merck) was determined according to the β -carotene bleaching method (Marco, 1968; Al-Saikhan et al., 1995; Jayaprakasha et al., 2001; Shahidi et al., 2001; Kaur and Kapoor, 2002) with the following modifications: a solution of β -carotene (Sigma, Steinheim, Germany) was prepared by dissolving 2 mg of β -carotene in 10 ml of chloroform; 1 ml of this solution was then pipetted into a round-bottom rotary flask containing 20 mg of linoleic acid (Sigma) and 0.2 g of Tween 20 (Sigma Chemical Co., St. Louis, MO, USA). After removing the chloroform under vacuum using a rotary evaporator at 30 °C, 50 ml of aerated distilled water was added to the flask with manual shaking. Aliquots (5 ml) of this prepared emulsion were transferred into tubes containing 0.2 ml of extracts or butylatedhydroxyanisole (BHA, 50 mg l⁻¹) or butylatedhydroxytoluene (BHT, 50 mg l⁻¹) or α -tocopherol (50 mg l⁻¹), which were used for comparative purposes. The control consisted of 0.2 ml of methanol instead of the extract. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470

nm. The samples were then subjected to thermal autoxidation at 50 °C in a water bath. Subsequent absorbance readings were recorded at 15 min intervals until the color of the β -carotene in the control sample had disappeared (105 min). The extent of inhibition of the absorbance is related to the concentration of antioxidant compounds. All samples were assayed in triplicate. The degradation rate of extracts was calculated according to zero order reaction kinetics. Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation:

$$AA = [1 - (A_t - A_0) / (A'_t - A'_0)] \times 100$$

A_0 : measured absorbance value of sample at zero time

A_t : measured absorbance value of sample after incubation (105 min) at 50 °C

A'_0 : measured absorbance value of control at zero time

A'_t : measured absorbance value of control after incubation (105 min) at 50 °C.

Determination of total phenolic content

Total phenolic contents were determined according to Tanner and Brunner (1979) and Kaur and Kapoor (2002). To 0.5 ml of methanolic solution of extracts, 7 ml of distilled water and 0.5 ml of Folin Ciocalteu reagent (Merck) were added and mixed well. After 3 min, 2 ml of 20% sodium carbonate was added and mixed well again. Absorbance of the resultant solution was read at 720 nm, after 1 h standing in a water bath at 25 °C. The results were expressed as mg of catechin l⁻¹ of fresh weight material using a standard calibration curve of (+)-catechin (Fluka Chemie, Switzerland).

Determination of flavonoids

A slightly modified version of the method of Zhishen et al. (1999) and Dewanto et al. (2002) was used to determine flavonoid contents of samples. One milliliter of extract was placed in a 10 ml volumetric flask, and 5 ml of distilled water and 0.3 ml of 5% NaNO₂ (Merck) were added and mixed. After 5 min, 0.6 ml of 10% AlCl₃.6H₂O (Merck) was added. Two milliliters of 1 mol l⁻¹ NaOH (Merck) was added 5 min later and then the volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured immediately at 510 nm. Flavonoid contents were calculated using a standard calibration curve, prepared from (+) catechin.

Statistical Analysis

All data analyses were performed by using the statistical software program MINITAB (Release, 13.0).

Results and Discussion

The antioxidant activity of fruits is shown in Table 1. It can be seen that different fruits exhibited varying degrees of antioxidant capacity. The average antioxidant activities of pomegranate, quince, grape, apple and pear were 62.7%, 60.4%, 26.6%, 25.7% and 13.7%, respectively. The bleaching of β -carotene in the presence of pomegranate, quince, apple, pear and grape is shown in Figure 1. Minimal bleaching of β -carotene emulsion occurred in samples treated with pomegranate followed by quince, whereas maximum bleaching of β -carotene emulsion was determined with pear. For comparative purposes, 50 mg l⁻¹ of BHT, BHA and α -tocopherol were also used and the antioxidant activities thereof were determined as 90.7, 92.4 and 90.9, respectively.

The different antioxidant activities of the fruits can be ascribed to their total phenolic concentrations. On comparing the data shown in Table 1, quince (3326 mg kg⁻¹) had much higher total phenolics than any of the other fruits studied, approximately 9, 3 and 3 times higher than those of pear (381 mg kg⁻¹), apple (986 mg

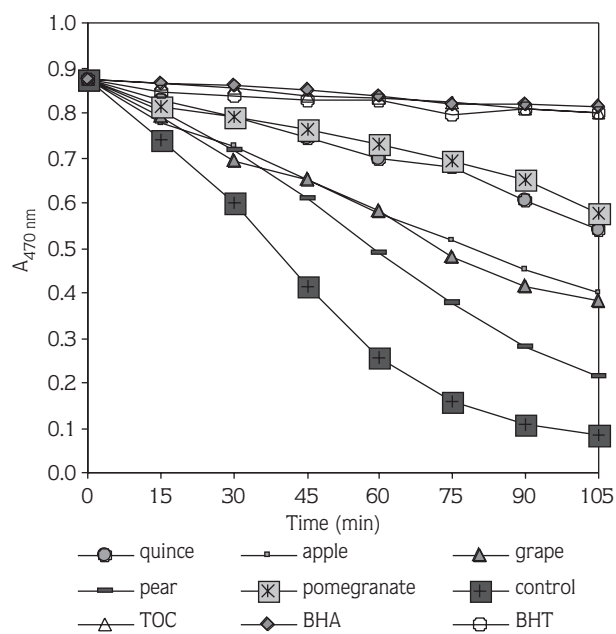


Figure 1. Antioxidant capacity of different fruits.

Table 1. Antioxidant activity, total phenolics and flavonoid content of selected fruits and vegetables. AA: Antioxidant activity.

Fruits and vegetables	Species	Variety	AA (%) ^a	Total Phenolics (mg kg ⁻¹)* ^a	Flavonoids (mg kg ⁻¹)* ^a
FRUITS					
Apple (<i>Malus domestica</i> B.)		Amasya	24.8 ± 0.6	1078 ± 38.9	730 ± 165.0
		Arapkızı	40.2 ± 3.5	1232 ± 12.0	823 ± 103.2
		Cooper	14.7 ± 3.5	876 ± 21.2	545 ± 106.1
		Gloster	20.8 ± 5.8	571 ± 21.2	308 ± 0.0
		Golden Delicious	20.7 ± 4.4	1146 ± 106.1	700 ± 106.1
		Granny Smith	24.2 ± 3.9	541 ± 23.3	282 ± 85.2
		Rome Beauty	40.7 ± 0.9	1110 ± 21.2	738 ± 225.0
		Starking	19.5 ± 3.5	1333 ± 3.5	778 ± 91.4
Grape (<i>Vitis vinifera</i> L.)		Müşküle	37.6 ± 2.3	2025 ± 56.6	1069 ± 45.6
		Seedless	15.6 ± 1.9	548 ± 17.7	452 ± 155.0
Quince (<i>Cydonia vulgaris</i>)		Çiçek dağı	65.1 ± 1.1	3093 ± 3.5	1676 ± 180.0
		Çubuk	52.3 ± 4.7	3103 ± 24.7	1735 ± 207.0
		Çukurgöbeği	68.2 ± 0.8	4306 ± 6.4	2115 ± 92.6
		Ekmek	61.5 ± 4.9	3763 ± 152.0	1723 ± 337.0
		Eşme	57.5 ± 6.4	2823 ± 223.0	1198 ± 51.8
		Kalecik	63.9 ± 1.7	3350 ± 14.1	1636 ± 500.0
		Kirli	51.4 ± 3.7	3140 ± 106.1	1510 ± 335.0
		Yerköy	63.2 ± 1.7	3033 ± 3.5	1456 ± 355.0
Pear (<i>Pyrus communis</i> L.)		Ankara	16.7 ± 2.6	345 ± 14.1	321 ± 6.0
		Deveci	13.0 ± 0.9	326 ± 7.1	321 ± 6.0
		Santa Maria	11.5 ± 2.8	473 ± 46.0	381 ± 108.9
Pomegranate (<i>Punica granatum</i> L.)			62.7 ± 2.1	2408 ± 38.9	459 ± 67.0
VEGETABLES					
Potato (<i>Solanum tuberosum</i>) Onion (<i>Allium cepa</i>) Spring onion (<i>Allium fistulosum</i>) Red radish (<i>Raphanus sativus</i>) Red cabbage (<i>Brassica oleracea</i>)		Potato (<i>Solanum tuberosum</i>)	14.2 ± 2.3	553 ± 102.5	153 ± 38.2
		Onion (<i>Allium cepa</i>)	12.5 ± 2.7	536 ± 113.1	170 ± 60.8
		Spring onion (<i>Allium fistulosum</i>)	15.7 ± 1.9	948 ± 130.8	329 ± 140.2
		Red radish (<i>Raphanus sativus</i>)	29.4 ± 0.4	1056 ± 106.8	179 ± 41.0
		Red cabbage (<i>Brassica oleracea</i>)	40.8 ± 3.7	2166 ± 7.1	842 ± 19.1
ANTIOXIDANTS					
BHT (50 mg l ⁻¹) BHA (50 mg l ⁻¹) α-Tocopherol (50 mg l ⁻¹)		BHT (50 mg l ⁻¹)	90.7 ± 0.9		
		BHA (50 mg l ⁻¹)	92.4 ± 1.1		
		α-Tocopherol (50 mg l ⁻¹)	90.9 ± 1.6		

^a Values are average ± SD for 2 replications

*Results are expressed as mg of (+) catechin per kg of fresh material

kg⁻¹) and grape (1287 mg kg⁻¹), respectively. Antioxidant activity and total phenolics in fruits showed a high correlation ($r^2 = 0.9307$, $P < 0.01$), implying that the phenolic compounds were major contributors to the observed antioxidant activity. On the other hand,

pomegranate (2408 mg kg⁻¹) contained a lower level of total phenolics than did quince, but its antioxidant activity was higher. Since pomegranate is known to be a good source of anthocyanins (Du et al., 1975), it can be suggested that these compounds accounted for its

antioxidant activity. Anthocyanins, a glycosylated form of anthocyanidins, are a group of flavonoids mostly responsible for the color of fruits from red through purple to blue. The main anthocyanins reported as being present in pomegranate are cyanidin glycosides (Du et al., 1975), the most abundant anthocyanins in fruits (Robards et al., 1999). Because of their *o*-dihydroxy structure, cyanidin glycosides have a high antioxidant capacity (Rice-Evans and Miller, 1998). Therefore, the higher antioxidant activity of pomegranate can be attributed to its cyanidin glycosides content.

Since it is known that flavonoids, a large group of phenolic compounds, have antioxidant activity (Middleton and Kandaswami, 1986), the flavonoid content of selected fruits was also determined. Average flavonoid contents of fruits were, in descending order, quince (1631 mg of catechin kg⁻¹), grape (761 mg of catechin kg⁻¹), apple (613 mg of catechin kg⁻¹), pomegranate (459 mg of catechin kg⁻¹) and pear (341 mg of catechin kg⁻¹). It was observed that the antioxidant activity of the fruits was also correlated with the flavonoid content ($r^2 = 0.8316$, $P < 0.01$). As can be seen in Table 1, the antioxidant activity of fruits increased with increasing flavonoid concentrations, with the exception of pomegranate. The flavonoid content of pomegranate was similar to that of pear, which has the lowest antioxidant capacity. As indicated above, the antioxidant ability of pomegranate is mostly attributed to anthocyanins. On the other hand, the low activity of pear may result from the presence of phenolic acids rather than flavonoids, since hydroxycinnamate-rich in fruits are considered to exhibit low antioxidant activity (Proteggente et al., 2002).

A difference in antioxidant activity was also observed among fruit cultivars. Apples showed antioxidant activity over a wide range, from 14.7% to 40.7%. It was noted that Rome Beauty (40.7%) and Arap Kızı (40.2%) with dark red skins, had higher antioxidant activity than did the other apple varieties. The higher antioxidant activity of these varieties is mostly attributed to anthocyanins, located in the skins of apples, which are mainly responsible for their intense red color. Pearson et al. (1999) also determined that anthocyanins are the main phenolic compounds present in the peel of red apples. The antioxidant activities of quince varieties were also similar in the range of 51.4% to 68.2%. Among pear varieties, Santa Maria demonstrated the lowest antioxidant activity even though its total phenolics and flavonoid contents

were high. This result may be attributed to the individual phenolic compounds of pears, which exhibit different antioxidant capacities.

Antioxidant activities of vegetables are also given in Table 1. Red cabbage showed the maximum inhibition of β -carotene oxidation (Figure 2). Höner and Cervellati (2002) also determined that red cabbage exhibits the highest antioxidant activity, using the Briggs-Rauscher reaction method. Among the other vegetables, red radish had the highest antioxidant activity (29.4%), followed by spring onion (15.7%), potato (14.2%) and onion (12.5%). Similar to our results, Al-Saikhan et al. (1995) determined that onion was much more active in the bleaching of β -carotene emulsion than was potato.

Red cabbage possessed the highest antioxidant activity, as well as the highest total phenolic concentration. The significant correlation ($r^2 = 0.9361$, $P < 0.05$) between antioxidant activity and total phenolic contents of vegetables was also confirmed by this result. In fruits and vegetables many researchers have also reported a statistically significant relationship between total phenolics and antioxidant activity (Kalt et al., 1999; Connor et al., 2002; Kaur and Kapoor, 2002; Moyer et al., 2002).

The correlation between flavonoid content and antioxidant potential of vegetables was not significant ($r^2 = 0.7975$, $P < 0.05$). This result may due to the limited number of vegetable samples under investigation.

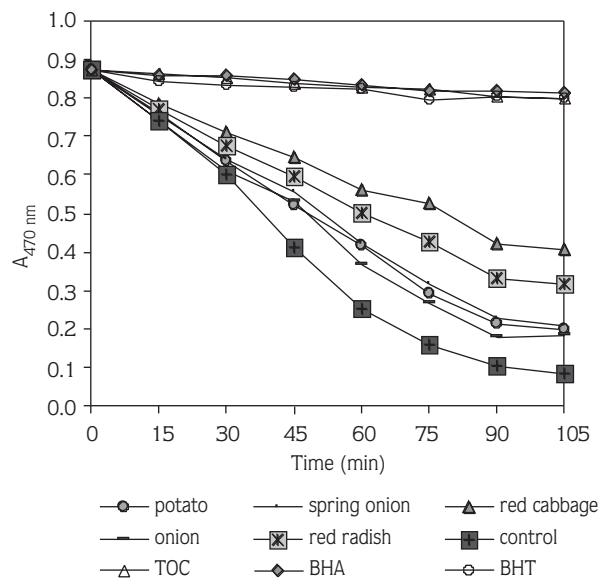


Figure 2. Antioxidant capacity of different vegetables.

However, it is known that vegetables generally contain much lower amounts of flavonoids, with the exception of onions, in comparison with fruits (Heinonen, 2002). On the other hand, Howard et al. (2000) reported that flavonoid concentrations were negatively correlated to the antioxidant activity of pepper cultivars. Red cabbage (Bridle and Timberlake, 1997) and red radish (Giusti and Wrolstad, 1996) are regarded as the most notable sources of anthocyanins, and so their higher antioxidant activity may be due in large part to the presence of anthocyanins.

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Conclusion

In fruits, the highest antioxidant activity was observed in pomegranate followed by quince, grape, apple and pear. Among vegetables, red cabbage exhibited the highest antioxidant activity followed by red radish, spring onion, potato and onion. The results indicated that total phenolics are the major contributor to the antioxidant activity of fruits and vegetables. In addition, the flavonoid content of fruits correlated closely with their antioxidant activity. The insignificant relationship between antioxidant activity and flavonoid content of vegetables may be attributed to the limited number of samples tested. As can be seen in red radish, red cabbage and pomegranate, the results suggest that anthocyanins can be considered the main source of antioxidant activity.

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