

Antioxidant capacity of juice from different papaya (*Carica papaya* L.) cultivars grown under greenhouse conditions in Turkey

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Abstract: The fruits of *Carica papaya* L. (Caricaceae) are valuable as food and are also used in traditional medicine. The present study was designed to assess the antioxidant potential of the juices of 3 papaya cultivars (PCJ): Sunrise Solo, Red Lady, and Tainung. The antioxidant capacity of PCJ obtained from fully ripened fruit was determined by the following methods: scavenging of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power assay, scavenging of superoxide radicals, 2-deoxyribose oxidation assay, and thiocyanate assay. Total phenolic content (TPC) of PCJ was determined by the Folin-Ciocalteu method. In the DPPH assay (IC₅₀ values), the effect of Sunrise Solo (52.1 ± 1.5 mL PCJ/g DPPH) was better than that of the Red Lady (63.4 ± 2.9 mL PCJ/g DPPH) and Tainung juices (71.8 ± 1.8 mL PCJ/g DPPH). PCJ showed a linoleic acid peroxide scavenging activity that was 5 times higher (during 72 h) than in the negative control but close to that of vitamin E, which served as the positive control. These PCJ antioxidant activities were significantly different from each other (P < 0.05). The tested reducing power activity, superoxide anion scavenging activity, and hydroxyl radical scavenging activity were found to follow the order of Sunrise Solo > Red Lady > Tainung, depending on their concentrations. This study demonstrated that different papaya cultivars have different antioxidant capacities and TPC amounts. Significant correlations were found between antioxidant capacity and TPC, indicating that phenols contribute to antioxidant capacity.

Key words: *Carica papaya*, cultivars, antioxidant, total phenolic content

Türkiye’de serada yetiştirilen üç farklı papaya (*Carica papaya* L.) çeşidinden elde edilen meyve suyunun antioksidan kapasitesi

Özet: Değerli bir meyve olan *Carica papaya* halk arasında tedavi amaçlı olarak da kullanılmaktadır. Bu çalışmada; Sunrise Solo, Red Lady ve Tainung olarak isimlendirilen papaya çeşitlerinden elde edilen meyve sularının (PJS) antioksidan kapasitelerinin açıklanması planlanmıştır. Tamamen olgunlaşmış meyvelerden elde edilen meyve sularının antioksidan kapasiteleri, 1,1-diphenyl-2-picrylhydrazyl (DPPH) serbest radikalini süpürme, indirgeyicilik gücü, süperoksit radikali süpürücü etkisi, 2-deoksiriboz oksidasyonu testi, ve tiyosiyanat model sistemleriyle açıklanmıştır. Toplam fenolik içerik (TPC) Folin-Ciocalteu metoduyla ölçülmüştür. DPPH radikalinin %₅₀ sinin inhibisyonunda (IC₅₀), Sunrise Solo’nun etkisi (52,1 ± 1,5 ml PCJ/g DPPH), Red Lady (63,4 ± 2,9 ml PCJ/g DPPH) ve Tainung’dan (71,8 ± 1,8 ml PCJ/g DPPH) daha iyi bulunmuştur. PJS, negative kontrol ile karşılaştırıldığında beş kat daha fazla, fakat pozitif kontrol olarak kullanılan E vitaminine yakın, linoleik asit peroksitlerini süpürme aktivitesi (72 saat boyunca) göstermiştir. PCJ’lerin bu antioksidan aktiviteleri birbirlerinden önemli ölçüde farklıdır (P < 0,05). İndirgeyicilik gücü, süperoksit anyon süpürme aktivitesi, hidroksil radikali süpürme aktivitesi Sunrise Solo > Red Lady > Tainung (konsantrasyonlarına bağlı) olarak sıralanmıştır. Bu çalışma, çeşitli papaya kültürlerinin farklı TPC ve antioksidan kapasiteye sahip olduklarını göstermektedir. Fenollerin antioksidan kapasiteye katkıda bulunduğunu göstererek, antioksidan kapasite ile TPC miktarı arasında önemli ilişki olduğu ortaya koymuştur.

Anahtar sözcükler: *Carica papaya*, kültürler, antioksidan, toplam fenolik içerik

Introduction

Natural antioxidants such as flavonoids, tannins, coumarins, xanthenes, phenolics, terpenoids, ascorbic acid, and proanthocyanins are found in various plant products, including fruits, leaves, seeds, oils, and juices (1-5). *Carica papaya* L. (Caricaceae) is cultivated in most subtropical and tropical countries for its fruits and its proteolytic enzymes. Papaya fruits contain components that can increase the total antioxidant power in blood and reduce the lipid peroxidation level. These components include α -tocopherol, ascorbic acid, beta carotene, flavonoids, vitamin B1, and niacin (6).

Overexpression of antioxidant enzymes or supplementation of some antioxidants appears to be effective in extending life-span in mouse models (7). Energy restriction, which can extend life-span, is also effective in reducing oxidative stress. However, in the attempt to demonstrate the antioxidant and antiaging effects of antioxidant supplementation, inconsistent data have been generated from animal and human studies (8-10). Therefore, more controlled studies are needed to test the beneficial effects of antioxidants occurring naturally in various food sources and to elucidate the possible underlying mechanisms. Biological properties of edible fruits can vary depending on stage of ripeness, whether the fruit is fresh or dried, storage conditions, and the fruit part considered (seed, peel, pulp, etc.) (11-15). The methanol extract of 12 tropical fruits from southern Florida, USA, including green papaya (14), as well as the juices of 8 pomegranate cultivars, dried papaya, and orange (5,16,17) were analyzed for antioxidant activity and total phenolics. Papaya is grown mainly in open-field conditions. Until now there have been no reports demonstrating the antioxidant capacity of papaya cultivars grown in greenhouses. The objectives of this study were: 1) to determine the antioxidant capacity of 3 papaya juices using 5 different spectrophotometric methods; 2) to compare the antioxidant capacity of Sunrise Solo, Red Lady, and Tainung papaya cultivars; and 3) to test the correlation between total phenolic contents (TPC) and antioxidant capacity.

Materials and methods

Juice preparation

The fruit was collected in May 2007 from the experimental field at the Seed Research and Development Center of Akdeniz University. The fruit was harvested at a commercially ripe stage in May, when about one-fourth of the peel color approached yellow. The juice was extracted from the homogenized flesh of the fruits of each cultivar. The juice was separated from the pulp by pressing it several times and centrifuging it at $1500 \times g$ for 20 min. The supernatant juice was filtered and kept at 4 °C until use (18). The weight of the fresh juice was 106.07 mg/mL (w/v). Fresh juices were analyzed for their antioxidant capacity and TPC. All juices had the same density. Nitrogen was used to prevent oxidation.

Total phenolic content (TPC)

The modified Folin-Ciocalteu method of Waterman and Mole (1994), with an incubation time of 120 min for color development, was used (19). Results are expressed as mg gallic acid equivalent (GAE)/100 g fresh sample.

Papaya cultivar juice (PCJ) was assessed for antioxidant capacities using the following 5 assays.

2,2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH assay was carried out following the method reported by Burits et al. (2000). Various amounts of the samples dissolved in methanol were added to 5 mL of a 0.004% methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl, free radical). After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm (20). Butylated hydroxyl toluene (BHT) and vitamin C were used as positive controls. Percent inhibition of free radical DPPH (I %) was calculated as follows:

$$I \% = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100.$$

Thiocyanate method

The antioxidant activity of PCJ was determined by the thiocyanate method (21). From each juice, 1 mL was taken and added to linoleic acid in a potassium phosphate buffer (2.5 mL, 0.04 M, pH 7.0). The mixed solution was incubated at 37 °C in a glass flask. The peroxide value was determined by reading the absorbance at 500 nm after reaction with FeCl_2 and

thiocyanate at several intervals during the incubation period. The solutions without added extracts were used as control samples. Vitamin E was used as a positive control.

Reducing power assay

The reducing power was determined according to the methods of Oyaizu (1986). Each sample (2.5, 5.0, and 10 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium phosphate buffer. The mixture was incubated at 50 °C for 20 min; then 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at $20 \times g$ for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank (22). Vitamin C was used as a positive control.

Superoxide anion scavenging activity

Measurement of the superoxide anion scavenging activity of PCJ was based on the method described by Liu et al. (1991). Superoxide radicals were generated in phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μ M) solution, 1 mL of NADH (78 μ M) solution, and sample solutions in water. The reaction was started by adding 1 mL of phenazine methosulfate (PMS) solution (10 μ M) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples (23). BHT and vitamin C were used as positive controls:

$$I \% = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100.$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the PCJ was assayed using the 2-deoxyribose oxidation method (24) with a slight modification. The reaction mixture contained 0.4 mL of 0.2 M sodium phosphate buffer (pH 7.4), 0.1 mL of sample solution of different concentrations, 0.1 mL of 1 mM EDTA, 0.1 mL of 1 mM FeCl_3 , 0.1 mL of 12 mM hydrogen peroxide, 0.1 mL of 60 mM 2-deoxyribose, and 0.1 mL of ascorbic acid in a tube. After incubation at 37 °C for

1 h, the reaction was stopped by adding 1 mL of 2% trichloroacetic acid and 1 mL of 1.0% thiobarbituric acid. The mixture was boiled for 15 min, cooled in ice, and extracted by *n*-butanol. The absorbance was measured at 532 nm against *n*-butanol (as a blank). The reaction mixture without a test sample was used as a control. BHT was a positive control:

$$I \% = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100.$$

All chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

The results of the replicates were pooled and expressed as mean \pm standard deviation. Analysis of variance and Student's *t*-test were carried out. Significance was set at $P < 0.05$ (25). All of the analyses were done in 5 repetitions for each sample concentration, and average values were calculated.

Results and discussion

In order to measure the antioxidant capacity of PCJ, 5 antioxidant methods, including DPPH, hydroxyl radical and superoxide anion scavenging activity, the thiocyanate method, and reducing power assay, were used. Phenolic compounds are called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide free radicals, and hydroxyl radicals. The correlation between phenol content and antioxidant capacity has been previously reported (26-28). The Folin-Ciocalteu method was used because many individual phenolic compounds that provide antioxidant activity in fruits cannot be identified or measured by high-performance liquid chromatography (HPLC) methods (29-31).

Total phenolic content (TPC)

TPCs (mg GAE/100 g) of the PCJs are shown in the Table. Sunrise Solo had a higher TPC (65 ± 1.9) than Red Lady (53 ± 1.6) and Tainung (41 ± 2.1). The TPC of PCJ was higher than that found in banana (14 ± 0.5) and apple juices (22 ± 2.8), while the TPC of fresh mango (71 ± 3.2), pomegranate (112.3 ± 6.4), strawberry (290 ± 5.9), and fresh guava (148 ± 8.4) juices was higher (8,15,32,33). The differences between the TPC of Sunrise Solo, Red Lady, and Tainung were found to be statistically significant (P

< 0.05). In other studies, the TPC of 8 pomegranate cultivars and 14 bayberry juice cultivars were found to vary between cultivars (16,32,33). Sunrise Solo had a higher TPC than some pomegranate cultivars and a lower TPC than some bayberry juices.

Antioxidant capacity

The free radical scavenging potential of PCJ at different concentrations was tested by the DPPH method. All cultivars had scavenging effects against DPPH radicals, ranging from 52.1-78.1 mL PCJ/g DPPH. The hierarchy for free radical scavenging activity with respect to IC₅₀ values was Sunrise Solo > Red Lady > Tainung (Table). The scavenging activity of Sunrise Solo was found to be higher than those of the other 2 cultivars, Red Lady and Tainung. Sunrise Solo had an IC₅₀ value of 52.1 mL of juice, while Red Lady and Tainung had IC₅₀ values of 63.4 and 71.8 mL of juice, respectively. The lower IC₅₀ values are associated with higher free radical scavenging activity (34). The effects of Sunrise Solo, Red Lady, and Tainung juices were no better than the positive controls in this assay (IC₅₀ values). When acidified aqueous methanol or 50% ethanol was used for homogenization of *C. papaya*, the IC₅₀ decreased (35,36). In another study, the free radical scavenging activity of pomegranate juices (PJ) obtained from 8 cultivars was determined by DPPH assay, showing different levels of activity among cultivars (16). Another study showed that bayberry juices from 14 cultivars had considerably different scavenging

activities and different amounts of TPC. The results also indicated that bayberry juices from red-colored cultivars possessed a higher phenolic compound content than juices from white-colored cultivars (37). The results obtained in another study showed that antioxidant activity tests were positively correlated with the total phenolic and flavonoid contents of the samples (38). According to our results, Sunrise Solo had the highest free radical scavenging activity of the 3 cultivars. The free radical scavenging activity of Sunrise Solo was higher than that of Iranian *Carica papaya* juice, unripe papaya, apple juice, orange juice, and some pomegranate cultivars such as I10, I23, and Zivzik; it was lower, however, than that of bergamot juice and blood orange juice (5,16,27,31,39). In another study in which free radical scavenging activity was measured by oxygen radical absorbance capacity (ORAC), papaya had higher activity than banana (12).

In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen by the PMS/NAD coupling reaction reduces NBT. The decrease in absorbance at 560 nm with antioxidants indicates the consumption of the superoxide anion in the reaction mixture (23). The superoxide anion scavenging activity of PCJ was concentration-dependent, and activity between cultivars was significantly different ($P < 0.05$). Sunrise Solo had the highest superoxide anion scavenging activity with the lowest IC₅₀ (22.4 mL) as compared to Red Lady (45.2 mL), Tainung (77.5 mL), and the positive controls.

Table. Antioxidant activity and total phenolic compounds of papaya cultivars.

Cultivars	Total phenolic content (mg GAE ² /100 g) ¹	IC ₅₀ (mL PCJ/g DPPH) ¹	IC ₅₀ (mL) (superoxide anion scavenging)	IC ₅₀ (mL) (hydroxyl radical scavenging)
Sunrise Solo	65 ± 1.9 ^a	52.1 ± 1.5 ^c	22.4 ± 1.6 ^a	12.56 ± 1.5 ^a
Red Lady	53 ± 1.6 ^{ab}	63.4 ± 2.9 ^{cd}	45.2 ± 1.4 ^c	22.19 ± 1.3 ^b
Tainung	41 ± 2.1 ^b	71.8 ± 1.8 ^d	77.5 ± 1.7 ^c	41.26 ± 1.6 ^c
BHT	-----	17.9 ± 1.1 ^b	32.6 ± 1.2 ^b	19.0 ± 1.2 ^{ab}
Ascorbic acid	-----	3.9 ± 0.1 ^a	49.6 ± 2.3 ^{cd}	Not determined

¹Mean ± SD obtained from analysis of 5 independent samples in duplicate; IC₅₀ values of positive controls (as µg/mL);

²GAE: gallic acid equivalent. Values that are followed by different letters within each column are significantly different ($P < 0.05$).

PCJ displayed some inhibition of the hydroxyl radical attack of deoxyglucose at different concentrations, a relevant endpoint for DNA protection against damage induced by free radicals. According to the 2-deoxyribose oxidation method results, PCJ had significantly different ($P < 0.05$) and concentration-dependent hydroxyl radical scavenging activity, as shown in the Table. Compared to the other 2 cultivars and the positive control, Sunrise Solo had the highest hydroxyl radical scavenging activity with the lowest IC_{50} (12.4 mL). Tainung had the lowest hydroxyl radical scavenging activity with the highest IC_{50} (46.9 mL). There was a positive correlation between TPC and free radical, hydroxyl radical, and superoxide anion scavenging activity of PCJ, and these had statistically different IC_{50} values ($P < 0.05$) (Table). According to our data, Sunrise Solo had higher activity than Red Lady and Tainung. Sunrise Solo also had high total chlorophyll a and b content compared to mutant papaya variety Golden (40). Chlorophyll may aid in the capture of radicals. The superoxide anion and hydroxyl radical scavenging activity of the flesh of unripe papaya was found to be higher than that of the seeds and the pulp. All parts of unripe papaya had higher activity than rice bran and lower activity than soybean paste and baker's yeast (11,14).

Total antioxidant activity in an emulsion of linoleic acid was measured by the ferric thiocyanate method. The ferric thiocyanate method measures the amount of peroxide, the primary product of oxidation, produced during the initial stages of oxidation. PCJ exhibited an antioxidant activity 5 times higher than that of the negative control. This was found to be close to that of vitamin E, which served as the positive control (Figure 1). The antioxidant activities of PCJ were statistically different from each other and from the negative control over 72 h ($P < 0.05$). Cultivar juices from the Ganesh variety and from 8 pomegranate cultivars showed different antioxidant activities (16,41). The antioxidant activity of 20 pomegranate cultivars was found to be between 15.59% and 40.72%, and these data demonstrated that the cultivar type was the main parameter influencing physicochemical properties and antioxidant activity in pomegranates (42). The antioxidant response of 3 cultivars of cucumber (*Cucumis sativus*) to salinity was found to be different (43).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (44). Therefore, the reductive capabilities in terms of $Fe^{+3} - Fe^{+2}$ transformations were measured in the presence of PCJ. PCJ had reducing activity against oxidation. At 2.5, 5.0, and 10 mg/mL concentrations, Sunrise Solo exhibited the maximum and Tainung the minimum reducing activity (Figure 2). Sunrise Solo had lower reducing activity than the positive control, ascorbic acid. The reducing power activity of papaya is lower than that of other fruits such as mangosteen, mango, and guava, but close to that of banana, as measured by ferric reducing antioxidant power (FRAP) (12).

Various cultivars may have different biological activity. The correlation between total phenol content and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables (4,32,45). According to our results, the rank of antioxidant capacity in the studied cultivars was Sunrise Solo > Red Lady > Tainung. There was a significant correlation between antioxidant capacity and total phenolics, indicating that phenolics contributed to the antioxidant activity. The highest antioxidant capacity was observed in Sunrise Solo, which also displayed a high content of total phenolics. This study indicated that various cultivars show different antioxidant capacities and amounts of phenolics. The broad range of activity of the juices suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of PCJ demonstrated in this study clearly indicates the potential application value of papaya fruits. The results seem to suggest that Sunrise Solo juice could exhibit antioxidant properties comparable to commercial synthetic antioxidants. Further studies on the isolation and characterization of individual compounds are needed to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds.

Statistically significant differences were observed among some papaya cultivars in terms of antioxidant capacity and TPC. The antioxidant capacity of papaya juices was influenced to a large extent by the type of cultivar. The results also suggested that

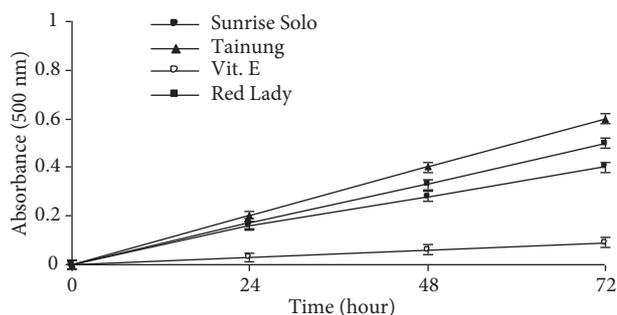


Figure 1. Antioxidant activity of Sunrise Solo, Red Lady, and Tainung cultivars of *Carica papaya* (L.).

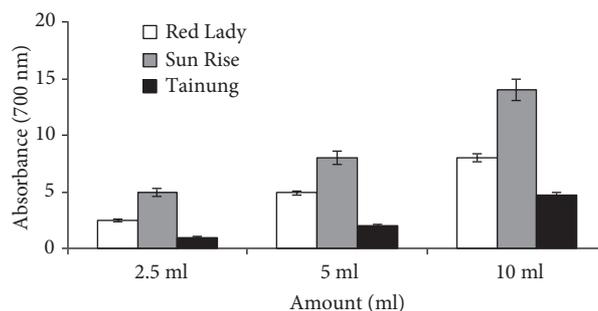


Figure 2. Reducing activity of Sunrise Solo, Red Lady, and Tainung cultivars of *Carica papaya* (L.).

antioxidant activities are concentration-dependent. PCJs were ranked as Sunrise Solo > Red Lady > Tainung, according to antioxidant capacity and TPC. The results showed a high correlation between those 5 antioxidant values and TPC.

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