

Antioxidant Activities and Anthocyanin Content of Fresh Fruits
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Fig fruit has been a typical component in the health-promoting Mediterranean diet for millennia. To study the potential health-promoting constituents of fig fruits, six commercial fig varieties differing in color (black, red, yellow, and green) were analyzed for total polyphenols, total flavonoids, antioxidant capacity, and amount and profile of anthocyanins. Using reversed-phase liquid chromatography (RP-LC), various concentrations of anthocyanins but a similar profile was found in all varieties studied. Hydrolysis revealed cyanidin as the major aglycon. Proton and carbon NMR confirmed cyanidin-3-O-rhamnoglucoside (cyanidin-3-O-rutinoside; C3R) as the main anthocyanin in all fruits. Color appearance of fig extract correlated well with total polyphenols, flavonoids, anthocyanins, and antioxidant capacity. Extracts of darker varieties showed higher contents of phytochemicals compared to lighter colored varieties. Fruit skins contributed most of the above phytochemicals and antioxidant activity compared to the fruit pulp. Antioxidant capacity correlated well with the amounts of polyphenols and anthocyanins ($R^2 = 0.985$ and 0.992 , respectively). In the dark-colored Mission and the red Brown-Turkey varieties, the anthocyanin fraction contributed 36 and 28% of the total antioxidant capacity, respectively. C3R contributed 92% of the total antioxidant capacity of the anthocyanin fraction. Fruits of the Mission variety contained the highest levels of polyphenols, flavonoids, and anthocyanins and exhibited the highest antioxidant capacity.

KEYWORDS: Common fig (*Ficus carica* L.); color; anthocyanins; flavonoids; polyphenols; antioxidant capacity; pulp; skin

INTRODUCTION

Ficus carica L., a deciduous tree belonging to the Moraceae family, is one of the earliest cultivated fruit trees. Today, fig is an important crop worldwide for dry and fresh consumption. On the basis of the Dietary Reference Intakes (DRI) data, published by the Food and Nutrition Board of the U.S. Institute of Medicine (1), and the nutrient composition of dried figs (2), they can be demonstrated to be a superior source of minerals and vitamins, providing per 100 g serving the following: iron, 30%; calcium, 15.8%; potassium, 14%; thiamin (B_1) 7.1%; and riboflavin (B_2) 6.2%. Figs are sodium free as well as fat and

cholesterol free (2, 3). Fig fruits contain at least 17 types of amino acids, among which aspartic acid and glutamine are the highest ones (3). Dried figs also contain relatively high amounts of crude fibers (5.8%, w/w), higher than those of all other common fruits (2). More than 28% of the fiber is of the soluble type, which has been shown to aid in the control of blood sugar and blood cholesterol and in weight loss. Dried figs also contain one of the highest concentrations of polyphenols among the commonly consumed fruits and beverages (2, 5).

Fig color varies from dark purple to green. Figs can be eaten whole and raw, but are often peeled; the flesh is eaten and the skin discarded. Although fig is one of the most abundant fruits in the Mediterranean diet, there is no information in recent scientific literature regarding its health-promoting potential. In a single paper, figs of an unknown variety were randomly picked at one time point and found to contain several carotenoids, including lutein, cryptoxanthin, lycopene, β -carotene, and α -carotene (4), with lycopene being the most abundant carotenoid, followed by lutein and β -carotene. The Mediterranean

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diet has been reported to promote health and quality of life in those who adhere to it, specifically by preventing pathophysiological conditions related to coronary heart disease and cancer (5). The high consumption of natural antioxidants, achieved by consuming fresh salads, vegetables, fruits, and their derived products, is generally considered to be a major beneficial contributor to the Mediterranean diet. Along with olive, fig (*F. carica* L.) is a characteristic and abundant fruit in this diet.

Many studies show that daily consumption of fruits and vegetables is associated with reduced risks for chronic degenerative diseases (6, 7). Fruits and vegetables contribute polyphenols as well as antioxidant vitamins to the diet. Some common fruits have high antioxidant capacities that cannot be accounted for by their vitamin C content (8). Eberhardt and others (9, 10) suggested that the major antioxidant activity in fruits and vegetables is due to the presence of polyphenol and flavonoid compounds. The antioxidant activity of several flavonoids, measured as scavenging of peroxyl radicals, is higher than that of vitamin E, vitamin C, or glutathione (11).

Anthocyanins extracted from purple-black rice showed 10–25 times higher antioxidant capacity than the equivalent concentrations of Trolox used as a reference antioxidant (12). In another study, the six common anthocyanidin standards and their glycosides potentially protected emulsified oil and human low-density lipoprotein (LDL) from oxidation, many showing capacities comparable to that of α -tocopherol, Trolox, catechin, or quercetin (13).

Berries such as blueberries, blackberries, and strawberries have high antioxidant capacities attributed to their high levels of polyphenols and anthocyanins (14, 15). Analyzing total antioxidant activity in various dietary plants, Halvorsen and co-workers showed that Smyrna figs from Turkey (honey color) had lower total antioxidant capacity than pomegranate, grape, or plum, but higher capacity than papaya, mango, apple, or banana (16).

The present study was designed to characterize the nutritional value of six commercial fig varieties with different colors. Within this framework, we determined the anthocyanin profile and amounts in these varieties and measured their total polyphenolics, flavonoids, and antioxidant capacity as well as their levels as a function of tissue of origin.

MATERIALS AND METHODS

Chemicals. Methanol and acetonitrile were purchased from BDH (Poole, U.K.). Phosphoric acid, ethyl acetate, and hydrochloric acid were purchased from BioLab Ltd. (Jerusalem, Israel). Aluminum chloride, sodium hydroxide, sodium carbonate, and sodium nitrite were purchased from J. T. Baker (Phillipsburg, NJ). Trolox, 2,2'-azinobis-(3-ethylbenzothiazolone-6-sulfonate) (ABTS), potassium persulfate, (+)-catechin, and gallic acid were purchased from Sigma (St. Louis, MO). Folin–Ciocalteu's phenol reagent was purchased from Fluka (Buchs, Switzerland). Cyanidin-3-*O*-Glu and cyanidin-3-*O*-rhamnogalucoside (cyanidin-3-*O*-rutinoside; C3R) were purchased from Apin Chemicals (Oxon, U.K.).

Extraction and Preparation of Crude Extracts. Main summer crop fruits from the commercial varieties Mission, Chechick, Bursa, Brown-Turkey, Brunswick, and Kadota were collected from a nethouse at The Volcani Center, Bet-Dagan, Israel. Fruits were collected at two different development stages: first, unripe fruits, prior to color break, and second, fully ripened and colored fruits. Skins were peeled and isolated from the flesh, weighed, and immediately immersed in chilled 0.1% (v/v) methanolic HCl to minimize air contact. The pigments were extracted by 5 min of maceration in a Waring blender containing 0.1% (v/v) methanolic HCl. The slurry was centrifuged at 10000 rpm, and the pigment-containing supernatant fluid was decanted. The extraction procedure was repeated four times. The supernatant fluids from several

Table 2. Gradient Program for RP-HPLC Analysis^a

time (min)	phosphate buffer (%)	acetonitrile (ACN) (%)	H ₃ PO ₄ (%)
0		98	2
5		98	2
8		92	8
10	76	11	13
24	68.5	11	20.5
25		98	2
30		98	2

^a Linear gradient for elution of anthocyanins from fig fruit extracts monitored at 520 nm.

extractions were combined and evaporated in vacuum to dryness at 25 °C. The residual pigment was resuspended in 2.0 mL of 0.1% (v/v) methanolic HCl. Samples were filtered through a 0.45 μ m cellulose syringe and stored at –20 °C until analysis.

Analysis of Fruit Skin Color. Fruit skin color was measured by a colorimeter (Chroma Meter CR-301, Minolta Co., Osaka, Japan), standardized with calibration plate sets CR-A47 and white plate of the fruit. Color parameters were expressed as tristimulus colorimetric measurements, that is, L^* , a^* , b^* , C , and H° . Negative L^* indicates darkness, and positive L^* indicates lightness. Negative a^* indicates green color, and high positive a^* indicates red color. High positive b^* indicates a more yellow color, and negative b^* indicates blue color. The chroma (C) value, calculated as $C = (a^{*2} + b^{*2})^{1/2}$, indicates color intensity or saturation. Hue angle, a parameter that has been shown to be effective in predicting visual color appearance, was calculated using the formula $H^\circ = \tan^{-1}(b^*/a^*)$, where 0° or 360° = red-purple, 90° = yellow, 180° = green, and 270° = blue (17). Color was measured at three random positions.

Determination of Polyphenols. The total polyphenolic content was determined with Folin–Ciocalteu reagent according to the method modified from Singleton (18) using gallic acid as a standard. Total fig phenolics extract was expressed on a fresh weight basis as milligrams of gallic acid equivalence (GAE) per 100 g of fresh weight (FW) or tissue. Samples of each extraction were analyzed in triplicate.

Determination of Flavonoids. The total flavonoids content was determined colorimetrically as described previously by Kim and others at 510 nm (19). The flavonoid content was determined by a (+)-catechin standard curve and expressed as mean of milligrams of (+)-catechin per 100 g of FW of fruit or tissue. Triplicate samples were analyzed for each sample.

Determination of Anthocyanins. Total anthocyanins content was determined according to the pH differential method (19). Absorbance was measured at 520 and 700 nm and expressed as cyanidin-3-glycoside (molar extinction coefficient of 26900 L·m⁻¹·mol⁻¹ and molecular weight of 449.2) equivalents per 100 g of FW of fruit or 100 g of tissue. Data are reported as means \pm SD for at least three replications.

Purification of Anthocyanins. The method described by Skrede and others (20) for the separation anthocyanins from other phenolics was followed. Briefly, fig extract was applied to a C₁₈ Sep-Pak cartridge that had been previously activated with ethyl acetate, followed by acidified methanol (0.01% HCl v/v) and acidified water (0.01% HCl v/v). Anthocyanins and other polyphenols were adsorbed onto the Sep-Pak column while sugars, acids, and other soluble compounds were removed by washing the cartridge with 2 volumes of acidified water (0.01% HCl v/v). The cartridge was dried with a current of nitrogen for 3 min. Less polar polyphenols were subsequently eluted with ethyl acetate. Then, anthocyanins were eluted with acidified methanol (0.01% HCl v/v). The acidified methanol solution was evaporated to near dryness and later lyophilized. The dry fraction was taken up in deionized water. Samples were filtered through a 0.45 μ m filter before analysis.

Reversed-phase liquid chromatography (RP-LC) was used to determine the profile of anthocyanins (Table 2). The HPLC system (Thermo Separation Products, Riviera Beach, FL) consisted of an autosampler (AS3000), an injector (100 μ L), a column oven (30 °C), a pump (P3000), and a diode array detector (UV6000). A reversed-phase (RP) C₁₈ column (250 mm \times 4.6 mm, Goldsil, Teknokroma,

Table 3. NMR Spectroscopic Data of the Major Anthocyanin Isolated from the Extract of Fig (*F. carica* L.) (δ in CD₃OD at 25 °C)

	¹ H	¹³ C
	δ	δ
2		164.35
3		145.75
4	8.94	136.36
5		159.22
6	6.68	103.59
7		170.62
8	6.89	95.34
9		157.81
10		113.37
1'		121.33
2'	8.03	128.52
3'		117.56
4'		156.01
5'	7.02	147.56
6'	8.26	118.55
1''	5.28	103.64
2''	3.67	74.80
3''	3.56	78.14
4''	3.43	71.34
5''	3.73	77.55
6''	3.61	67.91
	4.07	
1'''	4.66	102.28
2'''	3.82	71.98
3'''	3.64	72.56
4'''	3.34	74.04
5'''	3.57	69.88
6'''	1.17	17.98

Barcelona, Spain) was employed. A linear gradient consisting of acetonitrile (ACN), phosphate buffer (50 mM, pH 2.6), and H₃PO₄ (200 mM, pH 1.5) was employed. Anthocyanins were monitored at 520 nm. All samples were filtered through a 0.45 μ m syringe filter before analysis.

Acid Hydrolysis. Anthocyanins were heated at 121 °C for 40 min in 2 M HCl. The hydrolyzed compounds were extracted with ethyl acetate (EtOAc). Next, the EtOAc extract was collected and evaporated under a nitrogen stream. The dry residue was dissolved in 0.1% (v/v) methanolic HCl and chromatographed by RP-HPLC.

Trolox Equivalent Antioxidant Capacity (TEAC). The total antioxidant capacity of fig fruits extracts was measured by the ABTS^{•+} radical cation decolorization assay involving preformed ABTS^{•+} radical cation as described previously by Kerem and others (21). All determinations were performed in triplicate.

NMR Analysis. The NMR spectra of C3R were obtained for a CD₃-OD solution containing TMS as internal reference in a Bruker DMX-300 instrument (300.1 and 75.5 MHz for ¹H and ¹³C, respectively). Full assignments of all proton and carbon signals (see Table 3) were aided by such two-dimensional (2D) techniques as COSY (¹H \times ¹H correlation) and HMQC and HMBC (¹H \times ¹³C one-bond and long-range correlations, respectively).

Statistical Analysis. All determinations were based on at least three independent replicate samples for each fruit. Results were analyzed by JMP IN statistical discovery software using one-way variance analysis (ANOVA). When a significant difference was obtained ($P < 0.05$), the Tukey–Kramer HSD test was used to compare each pair of means.

RESULTS

Color Properties of Fig Fruits. Fruits of six commercial fig varieties differing in color (Figure 1) were examined. Fruit ripening was assessed by measuring the total soluble solids in the fruit pulp (data not shown) and by fruit color and reflectance (Table 1). In general, unripe fruits appeared to be brighter than ripe ones, as reflected by a decrease in L^* with maturity. For example, lightness values of unripe and ripe Bursa variety were 55.7 and 25.3, respectively. In addition, unripe fruits showed

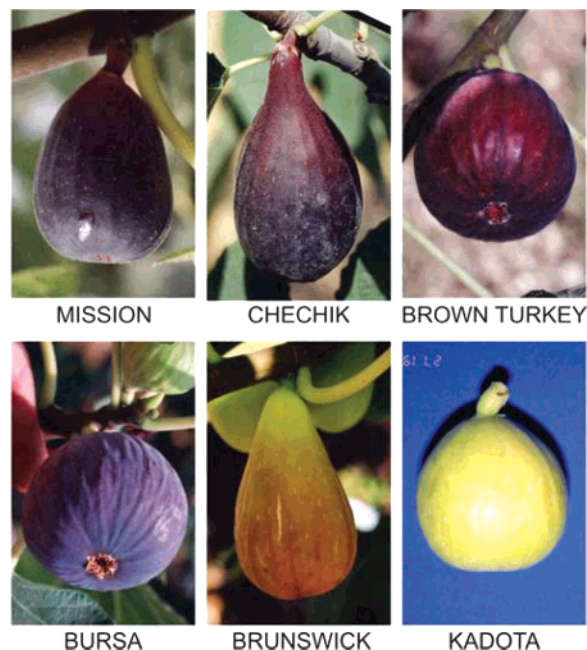
**Figure 1.** Color appearance of the six fig varieties examined in this study.**Table 1.** Color Properties of Ripe and Unripe Skins (Mean \pm SD)^a

fig variety	L^*	a^*	b^*	C	H°
(A) Ripe Skins					
Mission	23.0 \pm 1.4	1.4 \pm 0.6	0.3 \pm 0.1	1.4 \pm 0.6	12.0 \pm 3.2
Chechick	27.5 \pm 3.6	2.3 \pm 0.9	1.3 \pm 0.3	1.8 \pm 2.7	29.5 \pm 3.7
Bursa	25.3 \pm 3.2	8.8 \pm 1.1	3.3 \pm 0.5	9.4 \pm 1.4	20.7 \pm 0.3
Brown-Turkey	29.4 \pm 3.7	8.8 \pm 1.1	6.9 \pm 7.8	11.2 \pm 1.7	38.1 \pm 2.7
Brunswick	46.3 \pm 5.3	6.1 \pm 1.8	38.1 \pm 3.2	27.8 \pm 3.9	80.9 \pm 4.2
Kadota	66.9 \pm 7.4	-3.8 \pm 0.6	49.4 \pm 5.7	49.5 \pm 3.4	94.3 \pm 4.7
(B) Unripe Skins					
Mission	29.7 \pm 3.5	4.8 \pm 0.6	7.9 \pm 0.9	9.2 \pm 1.1	58.7 \pm 6.1
Chechick	30.6 \pm 3.7	2.9 \pm 7.5	11.1 \pm 0.3	7.6 \pm 0.9	75.4 \pm 3.8
Bursa	55.7 \pm 6.4	8.2 \pm 1.2	27.5 \pm 3.4	28.7 \pm 0.9	73.9 \pm 2.7
Brown-Turkey	49.4 \pm 5.6	2.2 \pm 0.9	12.3 \pm 3.1	12.5 \pm 3.2	79.9 \pm 2.7
Brunswick	74.4 \pm 8.4	-3.1 \pm 0.5	45.0 \pm 5.6	45.1 \pm 5.9	94.1 \pm 5.7
Kadota	67.6 \pm 7.2	-10.1 \pm 1.7	45.2 \pm 5.3	46.1 \pm 2.2	103.0 \pm 2.7

^a L^* , a^* , b^* , C , and H° are color parameters: lightness/darkness, red/green, blue/yellow, chroma, and hue angle, respectively.

higher color intensity (higher C value) than ripe ones. Changes in a^* and H° values for Bursa, Brown-Turkey, Brunswick, and Kadota varieties showed an alteration in color from green to red as maturity progressed. Minolta b^* values were similar for the different stages of maturity, but were reduced in the ripe, dark purple varieties (Chechick and Mission). Ripe fruits of Mission and Chechick varieties were characterized by dark purple color. Mission fruit surface showed lightness values (L^*) of 23.0 and hue angles (H°) of 12.0, whereas Chechick showed lightness values (L^*) of 27.5 and hue angles (H°) of 29.5, attesting to the latter's typical dark purple color. In addition, Mission was darker and purpler than Chechick. Bursa and Brown-Turkey varieties had L^* values of 25.3 and 29.4 and H° values of 20.7 and 38.1, respectively, indicating red-purple color. Values of L^* = 46.3 and H° = 63 were found for Brunswick, indicating a red-yellow color, whereas Kadota's green color was characterized by values of L^* = 66.9 and H° = 94.3. From these data we inferred that Kadota fruits appeared to be much brighter (larger L^* values) and more vivid (larger C) than the other varieties.

Total Anthocyanin Content in Ripe and Unripe Fig Varieties. Total anthocyanin level for unripe fruits ranged from 3.0 mg/100 g for Mission to 0.8 mg/100 g for Bursa and was

Table 4. Total Polyphenolics, Flavonoids, Anthocyanins, and TEAC Values of 100 g of Fig Fruits, Skins, and Pulps^a

fig type	total polyphenolics (mg of GAE/100 g)	total flavonoids (mg of (+)-catechin/100 g)	total anthocyanins (mg of cyn-3-glu/100 g)	TEAC (μ mol of TE/100 g)
(A) Fig Fruits				
Mission	281.1 \pm 3.0 a	21.5 \pm 2.7 a	10.9 \pm 1.3 a	716.3 \pm 52.6 a
Chechick	80.6 \pm 7.2 b	15.9 \pm 1.7 b	1.8 \pm 0.2 b	192.1 \pm 16.1 b
Brown-Turkey	58.1 \pm 6.3 bc	3.6 \pm 0.4 c	1.3 \pm 0.1 b	120.7 \pm 11.3 c
Bursa	56.0 \pm 6.2 c	2.7 \pm 0.3 c	0.3 \pm 0.03 c	100.3 \pm 8.6 c
Brunswick	50.0 \pm 4.6 c	2.3 \pm 0.3 c	nd	69.5 \pm 7.8 cd
Kadota	48.6 \pm 3.8 c	2.1 \pm 0.2 c	nd	25.0 \pm 3.1 d
(B) Fig Skins				
Mission	463.0 \pm 44.3 a	45.6 \pm 3.7 a	27.3 \pm 2.3 a	1987.0 \pm 175.4 a
Chechick	164.2 \pm 10.5 b	42.9 \pm 4.1 a	7.7 \pm 0.5 b	602.5 \pm 58.1 b
Brown-Turkey	141.1 \pm 12.4 bc	13.4 \pm 1.4 b	6.5 \pm 0.7 b	302.2 \pm 20.3 c
Bursa	123.0 \pm 13.4 c	10.1 \pm 1.3 b	4.1 \pm 0.3 c	292.5 \pm 24.6 c
Brunswick	65.5 \pm 5.6 d	3.8 \pm 0.5 c	0.7 \pm 0.1 d	101.2 \pm 7.8 d
Kadota	41.7 \pm 3.8 d	2.2 \pm 0.3 c	nd	82.0 \pm 5.1 d
(C) Fig Pulps				
Mission	100.6 \pm 8.6 a	5.7 \pm 0.5 a	0.3 \pm 1.3 a	357.5 \pm 30.6 a
Chechick	36.5 \pm 4.2 d	4.5 \pm 0.4 b	0.1 \pm 0.2 b	88.4 \pm 7.1 b
Brown-Turkey	42.9 \pm 4.7 d	1.6 \pm 0.2 d	0.1 \pm 0.1 b	79.3 \pm 6.3 b
Bursa	73.7 \pm 6.2 b	3.2 \pm 0.3 c	0.1 \pm 0.03 c	107.8 \pm 9.6 b
Brunswick	37.0 \pm 4.6 d	1.6 \pm 0.3 d	nd	36.5 \pm 2.8 c
Kadota	59.1 \pm 6.8 c	2.1 \pm 0.2 d	nd	20.8 \pm 3.1 c

^a Different letters in the same column indicate significant differences ($p < 0.05$); data expressed as means \pm standard deviations ($n = 3$) on a fresh weight basis. nd, not detectable; TE, Trolox equivalent.

low in the lighter varieties Brunswick and Kadota. Invariably, anthocyanins in unripe fruits accumulated in the fruit skin (data not shown). We next compared the anthocyanin level and tissue distribution in ripe versus unripe fig fruits. In general, total anthocyanin level increased in all ripe fruits compared with unripe ones. Among all fig varieties, the Mission variety showed the highest anthocyanin content, with most of the anthocyanins located in the fruit skin (**Table 4B**). The amount of anthocyanins in ripe fruits (**Table 4A**) ranged from 11.0 mg/100 g for Mission to 0.3 mg/100 g for Bursa, whereas Brunswick and Kadota varieties had lower anthocyanin contents of 0.1 mg/100 g. The Mission variety was 5 times richer in anthocyanins compared with the other dark variety, Chechick. The anthocyanin content for Brown-Turkey was significantly higher compared to that of the other purple variety, Bursa. The amounts of anthocyanins in the lighter varieties Brunswick and Kadota were the lowest, with no significant difference between them.

In both ripe and unripe fig fruits, fruit skins had higher anthocyanin levels (**Table 4B**) compared to pulps (**Table 4C**). For example, Mission fruit skin had 100 times higher anthocyanin content compared to pulp. Accordingly, dark fruit skins had more anthocyanins compared to purple or lighter varieties. The amounts of anthocyanins in ripe fruit skins ranged from 27.0 mg/100 g for Mission to 4.0 mg/100 g for Bursa, whereas Brunswick and Kadota fruit skins had lower anthocyanin levels.

Profile of Anthocyanins in Ripe Fruit Skins. The overall anthocyanin content is considered a differentiating mark for figs. Identical profiles were found for all samples. Four anthocyanins were resolved and observed at 520 nm by RP-LC (**Figure 2**). Acid hydrolysis of the two major anthocyanins (2, 3) and spiking with a commercial cyanidin standard suggested the aglycon to be the cyanidin in all fig varieties. The two major compounds were isolated by RP-LC for further spectral analysis and identification. By using authentic standard and by comparison with literature data, we were able to ascribe compound 2 as cyanidin-3-glu ($t_R = 18.5$ min, $\lambda_{max} = 280, 520$ nm). Compound 3, the dominant anthocyanin, was observed at $t_R = 18.8$ min with $\lambda_{max} = 254, 516$ nm. The $Abs_{440}/Abs_{\lambda_{max}}$ ratio values calculated for the dominant anthocyanin ranged from 31 to 32%,

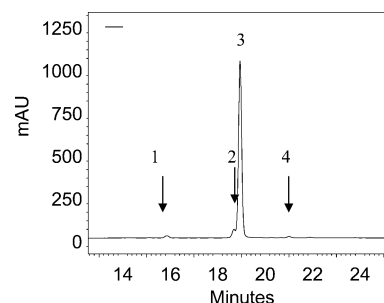
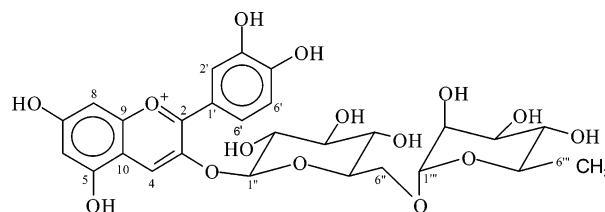


Figure 2. RP-HPLC chromatogram of Mission fruit extract. Absorbance at 520 nm is shown. The two major anthocyanins are cyanidin-3-glu (2) and cyanidin-3-rhamnoglucoside (C3R) (3).

Chart 1. Chemical Structure of Cyanidin-3-rhamnoglucoside (C3R)



indicating a substitution in the C-3 position of the flavylium ring (22). Notably, anthocyanins with glycosidic substitution at position 3 exhibit an absorbance ratio ($A_{400-440nm}/A_{520nm}$) that is almost twice as large as that of anthocyanins with glycosidic substitutions at position 5 or at both positions 3 and 5 (23). In addition, the obtained $Abs_{280}/Abs_{\lambda_{max}}$ (67–100%) and $Abs_{310}/Abs_{\lambda_{max}}$ (13–22%) ratio confirmed that the major anthocyanin was a simple anthocyanin molecule without acylation of glycoside with aromatic acids (24, 25). By using 1H and ^{13}C NMR (**Table 3**) the major anthocyanin was identified as C3R. The chemical structure is shown in **Chart 1**.

Total Flavonoids in Ripe Fig Fruits. Anthocyanins belong to the widespread class of phenolic compounds collectively named flavonoids (26). Total flavonoid content of the six fig varieties was measured colorimetrically and found to be significantly higher in the dark varieties compared to the lighter

ones (Table 4A), with most flavonoids located in the fruit skin (Table 4B). Total fruit flavonoid ranged from 21.5 mg/100 g of FW for Mission to 2.1 mg/100 g of FW for Kadota. The darker varieties, Mission and Kadota, had significantly higher flavonoid levels than other varieties. Also, the flavonoid content in Mission was significantly different from that of Chechick. Here, the purple and lighter varieties did not differ. Skin was the major tissue that contributed to total flavonoids content (Table 4B,C), ranging from 45.6 mg/100 of FW for Mission to 2.2 mg/100 g of FW for Kadota. There was a significant difference between the darker (Mission and Chechick) and purple (Brown-Turkey and Burssa) varieties and the lighter varieties (Brunswick and Kadota), but no significant difference was found between the similar color-group varieties.

Total Polyphenols in Ripe Fruits. Anthocyanins and flavonoids belong to the widespread class of polyphenolic compounds. Results showed that the dark varieties were richer in polyphenols (Table 4A), ranging from 281.0 mg/100 g of FW for Mission to 49 mg/100 g of FW for Kadota. There was a significant difference in polyphenols content between the dark varieties Mission and Chechick, but not between the purple (Brown-Turkey and Bursa) and the lighter varieties (Brunswick and Kadota).

Again, skins had higher polyphenols than pulps (Table 4B,C). Polyphenols content in skins varied from 463 mg/100 g of FW for Mission to 42 mg/100 g of FW for Kadota (Table 4B). Polyphenols content in pulps ranged from 100 mg/100 g of FW for Mission to 37 mg/100 g of FW for the lighter variety Brunswick. Differences in polyphenols contents between skin and pulp were higher in the dark varieties relative to the lighter varieties Brunswick and Kadota. In addition, polyphenols content in Kadota pulp (a green variety) was higher compared with skin. In general, Mission fig had significantly higher polyphenols content compared to the other varieties, with the skin being the major contributing tissue.

TEAC. In agreement with the above results, total antioxidant capacities were higher in extracts of dark fig varieties (Table 4A), ranging from 716 $\mu\text{mol}/100$ g of FW for Mission to 25 $\mu\text{mol}/100$ g of FW for Kadota. Mission variety had significantly higher antioxidant capacity compared with all other varieties tested including the other dark variety, Chechick. There was no significant difference between the purple varieties Brown-Turkey and Bursa and the yellow variety Brunswick. Furthermore, the skin was the major contributing tissue to the total antioxidant capacity compared to the pulp (Table 4B,C), having ≈ 3 -fold higher capacity (Table 4B,C). Thus, skin antioxidant capacity varied from 2000 $\mu\text{mol}/100$ g of FW for Mission to 82 $\mu\text{mol}/100$ g of FW for Kadota (Table 4B). Total antioxidant capacity of Mission was 3 times higher compared to Chechick. Results showed that purple skins of Brown-Turkey and Bursa were higher in antioxidant capacities compared with the two lighter varieties. In pulps, antioxidant capacity varied from 358 $\mu\text{mol}/100$ g of FW for Mission to 21 $\mu\text{mol}/100$ g of FW for Kadota. Antioxidant capacity of Mission pulps was 4 times higher than that of Chechick pulps. There was no significant difference between Chechick pulps and purple variety pulp. Antioxidant capacities of purple pulps were higher compared with those of the lighter pulps. There was no significant difference between pulps of the two lighter varieties.

Correlation between Antioxidant Capacity and Phytochemical Contents. A high correlation was demonstrated between either total polyphenols or total anthocyanins and

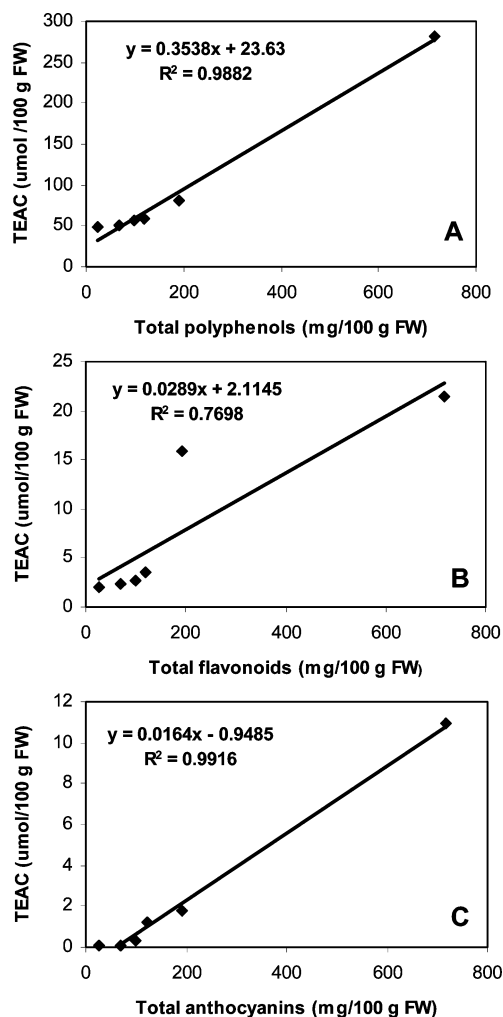


Figure 3. Relationship between total polyphenols content (A), total flavonoids content (B), total anthocyanins content (C), and antioxidant capacity (TEAC) of six fig varieties, expressed as Trolox equivalents per 100 g of FW of fruits.

antioxidant capacities, with $R^2 = 0.985$ and $R^2 = 0.992$, respectively (Figure 3A,C). The correlation coefficient for flavonoids was lower ($R^2 = 0.782$), but still significant (Figure 3B). Therefore, we measured the antioxidant capacity (TEAC) of the anthocyanin fraction. For this assay, we examined figs of Mission and Brown-Turkey. The relative antioxidant capacity for the anthocyanin fraction based on the TEAC assay was 28 and 36% for Brown-Turkey and Mission, respectively. C3R in fig skins contributed 92% of the total antioxidant capacity of the anthocyanin fraction.

DISCUSSION

In pigmented plant foods, as well as in plants and vegetables, color is produced by anthocyanins, chlorophylls, or carotenoids (15, 27). Anthocyanins belong to the widespread class of phenolic compounds, collectively named flavonoids. Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins (26). The attractiveness of fig fruits largely stems from their wide diversity of colors, ranging from dark purple to green. We examined the amounts of anthocyanins in six commercial varieties of figs. The dark variety Mission contained the highest levels of anthocyanins, whereas the yellow-fruited variety Kadota contained the lowest levels. In all figs tested, anthocyanins concentrated in the fruit skins and constituted the main coloring compounds.

Despite the wide color diversity among the different cultivars, our results show a similar anthocyanin profile in all tested fruits, with C3R accounting for 95% of the total. The identification of C3R was confirmed by ^1H and ^{13}C NMR analysis (see **Table 3** for a complete assignment of all the protons and carbons in the molecule). The ^1H NMR data we obtained are consistent with the literature (28); however, in that paper the authors provide only ^1H chemical shifts and only of the aglycon and anomeric protons. In addition, the reported structure of C3R contains an error in stereochemistry (at carbons 1'' and 2''). The correct chemical structure is shown in **Chart 1**.

We propose cyanidin to be the sole anthocyanidin skeleton in fig fruits, based on RP-LC and acid hydrolysis. Previously, Robinson and Robinson (29) and Puech and co-workers (30) assessed the composition of anthocyanins in fig. Robinson and Robinson suggested cyanidin-3-*O*-glucoside as the sole anthocyanin in fig. We could resolve four anthocyanins, of which cyanidin-3-*O*-glucoside was a rather minor component, whereas C3R was found to be the major pigment in the fruit skin. Using TLC and densitometry tests, Puech and co-workers suggested the presence of three anthocyanins in Mission fruits, namely, cyanidin-3-rhamnoglucoside, cyanidin 3,5-diglucoside, and pelargonidin 3-rhamnoglucoside, with cyanidin-3-rhamnoglucoside being the predominant pigment in ripe skins (30). This anthocyanin was observed also in fruits such as fresh olives (31), mulberries, cherries, and berries (32, 33), *Euterpe oleracea* Mart. (acai palm) (34), and black currant (*Ribes nigrum* L.) (35).

Differences in fruit color may result from differential expression of genes controlling the anthocyanin pathway, with the highest expression associated with the dark purple varieties. In a study involving berries that are genetically identical except for their anthocyanin content, the researchers suggested that a mutation in the anthocyanin biosynthetic pathway reduced their pigment content (36).

In addition to anthocyanins, other polyphenols including flavonoids are widely distributed in fruits and vegetables and may contribute to a phenomenon called copigmentation, which extends the spectrum of the resultant colors. The Mission variety is characterized by dark purple fruits, containing the highest levels of polyphenols and flavonoids among the tested varieties. The yellow-fruited variety, Kadota, has been found to contain low levels of polyphenols and flavonoids. The purple-red-fruited varieties, Brown-Turkey and Bursa, showed intermediate contents of anthocyanins, higher than that of Brunswick, which represented the yellow varieties. Similar to anthocyanins content, fruit skin also contained the highest contents of polyphenols and flavonoids.

Fruits and vegetables containing high concentrations of phytochemicals have attracted considerable interest in recent years due to their potential health-promoting effects. These effects are at least partly due to their antioxidant capacity (36, 37). The total amount of electron-donating antioxidants in the diet is derived from combinations of individual antioxidants that occur naturally in food (14). For instance, a high concentration of vitamin C may act adversely as pro-oxidant (38). In this study the TEAC assay was used to evaluate the antioxidant capacity of fruits of six fig varieties, and the obtained values were compared to the amounts of total polyphenols, flavonoids, and anthocyanins determined in each variety. The dark Mission variety contained the highest levels of polyphenols, flavonoids, and anthocyanins and exhibited the highest antioxidant capacity among the tested varieties. The yellow-fruited variety, Kadota, contained low levels of polyphenols, flavonoids, and anthocyanins and had low antioxidant activity. The purple-red fruited

variety Brown-Turkey showed intermediate contents of polyphenolic compounds, flavonoids, and anthocyanins and had higher antioxidant activity than Brunswick, which represented the yellow varieties. The data presented above show that the higher the polyphenols contents, especially anthocyanins, in fig fruits, the higher was their antioxidant capacity. We conclude that the darker fig varieties have higher antioxidant capacities. Bearing in mind the low levels of vitamin C in fig fruits, we presume that the major contribution to the antioxidant capacity of figs is derived from the various polyphenols. The results demonstrate a high correlation between total polyphenols or anthocyanins contents and the antioxidant capacity, but not with total flavonoids. Anthocyanins are in general the more water-soluble polyphenols in fruits. This may explain the better correlation demonstrated for the TEAC assay with anthocyanin content, as this assay measures reducing power in aqueous environment. In addition, we measured the relative antioxidant capacity of the anthocyanin fraction in extracts from ripe Mission and Brown-Turkey fruits. In these varieties, the relative contributions of anthocyanins to total antioxidant capacities were 36 and 28%, respectively. In another study, the contributions of the anthocyanins to the overall antioxidant capacities of the *E. oleracea* Mart. (acai) fruit were estimated to be $\approx 10\%$ (34). The relative antioxidant activities against radicals generated in aqueous phase was assessed by Rice-Evans et al. (39). The results show that compounds such as cyanidin with 3',4'-dihydroxy substituents in the B ring and conjugation between the A and B rings have antioxidant potentials 4 times that of Trolox, the vitamin E analogue. Kahkonen et al. (26) found that anthocyanidins lacking the *O*-diphenyl structure in the B ring (malvidin, pelargonidin, petunidin, and peonidin) had lower efficiency toward the DPPH radical compared to cyanidin and delphinidin. In addition, the antioxidant capacity of C3R was significantly higher than that of either cyanidin or cyanidin-3-glucoside.

Anthocyanins are known to produce high values of TEAC. The completely conjugated structure of anthocyanins allows electron delocalization, resulting in a very stable radical scavenging product, which is favorable in terms of antioxidative ability (26). This is in agreement with other studies suggesting that among all common fruits and vegetables in the diet, berries, especially those with dark blue or red colors, have the highest antioxidant capacity (36, 37). Moreover, in animal studies, consumption of dark berries was found to protect against peroxynitrite-induced endothelial dysfunction and chemically induced cancer (40, 41).

The average daily intake of anthocyanins per person has been estimated to be up to 200 mg (42). Mission is the richest fig variety in anthocyanins of the six varieties examined in this study, containing 11.0 ± 1.0 mg/100 g of FW. Because skins are shown here to be the major source of anthocyanins and polyphenols, the consumption of whole ripe fruits is recommended. For comparison, anthocyanin content in Oregon cranberries ranged from 65 to 589 mg/100 g of FW (8) and in Georgia-grown blueberries and blackberries the anthocyanins content ranged from 13 to 197 mg/100 g of FW (14).

The composition of phytochemicals such as carotenoids and of minerals and hence the health-promoting potential of fig fruits should be further examined. Our work focused on beneficial compounds present in fresh fig fruit. Following studies should also determine the properties of dried fruits.

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LITERATURE CITED

- (1) Vitamin and mineral recommendations; <http://www.nal.usda.gov/fnic/etext/000105.html#q2>.
- (2) Vinson, J. A.; Zubik, L.; Bose, P.; Samman, N.; Proch, J. Dried fruits: excellent *in vitro* and *in vivo* antioxidants. *J. Am. Coll. Nutr.* **2005**, *24*, 44–50.
- (3) Lianju, W.; Weibin, J.; Kai, M.; Zhifeng, L.; Yelin, W. The production and research of fig (*Ficus carica* L.) in China. *Acta Hortic.* **2003**, *605*, 191–196.
- (4) Su, Q.; Rowley, K. G.; Itsiopoulos, C.; O'Dea, K. Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. *Eur. J. Clin. Nutr.* **2002**, *56*, 1149–1154.
- (5) Vinson, J. A. The functional food properties of figs. *Cereal Foods World* **1999**, *4*, 82–87.
- (6) Dragsted, L. O.; Strube, M.; Larsen, J. C. Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacol. Toxicol.* **1993**, *72* (Suppl. 1), 116–135.
- (7) Genkinger, J. M.; Platz, E. A.; Hoffman, S. C.; Comstock, G. W.; Helzlsouer, K. M. Fruit, vegetable, and antioxidant intake and all-cause, cancer, and cardiovascular disease mortality in a community-dwelling population in Washington county, Maryland. *Am. J. Epidemiol.* **2004**, *160*, 1223–1233.
- (8) Wada, L.; Ou, B. Antioxidant activity and phenolic content of Oregon canberries. *J. Agric. Food Chem.* **2002**, *50*, 3495–3500.
- (9) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903–904.
- (10) Liu, R. H.; Eberhardt, M. V.; Lee, C. Y. Antioxidant and antiproliferative activities of selected New York apple cultivars. *N. Y. Fruit Q.* **2001**, *9*, 15–17.
- (11) Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303–311.
- (12) Jchikawa, H.; Ichianagi, T.; Xu, B.; Yoshii, Y.; Konishi, T. Antioxidant activity of anthocyanin extract from purple black rice. *J. Med. Food* **2001**, *4*, 211–218.
- (13) Kahkonen, P. M.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycons. *J. Agric. Food Chem.* **2003**, *51*, 628–633.
- (14) Sellappans, S.; Akoh, C. C.; Krewer, G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J. Agric. Food Chem.* **2002**, *50*, 2432–2438.
- (15) Rababah, T. M.; Ereifej, K. I.; Howard, L. Effect of ascorbic acid and dehydration on concentration of total phenolics, antioxidant capacity, anthocyanins and color in fruits. *J. Agric. Food Chem.* **2005**, *53*, 4444–4447.
- (16) Halverson, L. B.; Holte, K.; Myhrstad, C. M.; Barikmo, I.; Blomhof, R. A. Systematic screening of total antioxidant in dietary plants. *J. Nutr.* **2002**, *132*, 461–471.
- (17) Sapers, G. M. Color characteristics and stability of nonbleeding cocktail cherries dyed with carotenoid pigments. *J. Food Sci.* **1994**, *59*, 135–138.
- (18) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin–Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
- (19) Kim, D.; Jeong, S. W.; Lee, C. Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* **2003**, *81*, 321–326.
- (20) Skrede, G.; Wrolstad, R. E.; Drust, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci.* **2000**, *65*, 357–364.
- (21) Kerem, Z.; Bilkis, I.; Flaishman, M. A.; Sivan, L. Antioxidant activity and inhibition of α -glucosidase by *trans*-resveratrol, piceid, and a novel *trans*-stilbene from the roots of Israeli *Rumex bucephalophorus* L. *J. Agric. Food Chem.* **2006**, *54*, 1243–1247.
- (22) Giusti, M. M.; Rodriguez-Sasona, L. E.; Wrolstad, R. E. Molar absorptivity and color characteristic of acylated and nonacylated pelargonidin-based anthocyanins. *J. Agric. Food Chem.* **1999**, *47*, 4631–4637.
- (23) Harborne, J. B. *Comparative Biochemistry of the Flavonoids*; Academic Press: New York, 1976.
- (24) Woodall, G. S.; Stewart, G. R. Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? *J. Exp. Bot.* **1998**, *49*, 1447–1450.
- (25) Mozetic, B.; Trebse, P.; Haribar, J. Determination and quantification of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunus avium* L.) from Nova Gorica region (Slovenia). *Food Technol. Biotechnol.* **2002**, *40*, 207–212.
- (26) Kahkonen, P. M.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycons. *J. Agric. Food Chem.* **2003**, *51*, 628–633.
- (27) Roca, M.; Minguez-Mosquera, M. I. Changes in chloroplast pigments of olive varieties during fruit ripening. *J. Agric. Food Chem.* **2001**, *49*, 832–839.
- (28) Longo, L.; Vasapollo, G. Anthocyanins from bay (*Laurus nobilis* L.) berries. *J. Agric. Food Chem.* **2005**, *53*, 8063–8067.
- (29) Robinson, G. M.; Robinson, R. A survey of anthocyanins. *Biochem. J.* **1932**, *26*, 1647–1664.
- (30) Puech, A. A.; Rebeiz, C. A.; Catlin, P. B.; Crane, J. C. Characterization of anthocyanins in fig (*Ficus carica* L.) fruits. *J. Food Sci.* **1975**, *40*, 775–780.
- (31) Romero, C.; Brenes, M.; Garcia, P.; Garcia, A.; Garrido, A. Polyphenol changes during fermentation of naturally black olives. *J. Agric. Food Chem.* **2004**, *52*, 1973–1979.
- (32) Chen, P. M.; Chu, S. C.; Chiou, H. L.; Chiang, C. L.; Heish, Y. S. Mulberry anthocyanins cyaniding 3-rutinoside and cyanidin-3-glucoside, exhibited an inhibitory affect on the migration and invasion of a human lung cancer cell line. *Cancer Lett.* **2005**, *20*, 1–12.
- (33) Seeram, N. P.; Momin, R. A.; Nair, M. G.; Bourquin, L. D. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* **2001**, *8*, 362–369.
- (34) Lichtenthaler, R.; Rodrigues, R. B.; Maia, J. G.; Papagiannopoulos, M.; Fabricius, H.; Marx, F. Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (Acai) fruits. *Int. J. Food Sci. Nutr.* **2005**, *56*, 53–64.
- (35) McDougall, G. J.; Gordon, S.; Brennan, R.; Stewart, D. Anthocyanin-flavanol condensation products from black currant (*Ribes nigrum* L.). *J. Agric. Food Chem.* **2005**, *53*, 7878–7885.
- (36) Liu, M.; Li, Q. X.; Weber, C.; Lee, C. Y.; Brown, J.; Liu, R. H. Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem.* **2002**, *50*, 2926–2930.
- (37) Wu, X.; Pittman, H. E., 3rd; Prior, R. L. Fate of anthocyanins and antioxidant capacity in contents of the gastrointestinal tract of weanling pigs following black raspberry consumption. *J. Agric. Food Chem.* **2006**, *54*, 583–589.
- (38) Carr, H.; Frei, B. Does vitamin C acts as pro-oxidant under physiological conditions? *FASEB J.* **1999**, *13*, 1007–1024.
- (39) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22* (4), 375–383.
- (40) Talalay, P.; Fahey, J. W. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J. Nutr.* **2001**, *131*, 3027–3033.
- (41) Casto, B. C.; Kresty, L. A.; Kraly, C. L.; Weghorst, C. M. Chemoprevention of oral cancer by black raspberries. *Anticancer Res.* **2002**, *22*, 4005–4015.
- (42) Kuhnau, J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.* **1976**, *24*, 117–191.