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ARTICLE

Antioxidant Phytochemicals and Antioxidant Capacity of Biofortified Carrots (*Daucus carota* L.) of Various Colors

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Antioxidants and antioxidant capacity of seven colored carrots were determined. Five anthocyanins, chlorogenic acid, caffeic acid, and four carotenoids were quantified by HPLC. Total phenolic content was determined according to the Folin−Ciocalteu method. Antioxidant capacities of the hydrophilic and hydrophobic fractions were determined by using the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) methods. The relative antioxidant capacity index was determined. Anthocyanins were the major antioxidants in purple-yellow and purple-orange carrots, and chlorogenic acid was a major antioxidant in all carrots. Carotenoids did not contribute to total antioxidant capacity, but correlated with antioxidant capacity of hydrophobic extracts. Both the DPPH and ABTS assays showed that the hydrophilic extract had higher antioxidant capacity than the hydrophobic extract. Purple-yellow carrots had the highest antioxidant capacity, followed by purple-orange carrots, and the other carrots did not significantly differ. This information is useful for consumers and may help horticulturists develop carrots with higher antioxidant capacity.

KEYWORDS: Antioxidant capacity; anthocyanins; carotenoids; carrots; chlorogenic acid; total phenolic content

INTRODUCTION

Consumption of vegetables can lower the incidence and mortality rates of cancer and cardio- and cerebrovascular diseases (*1*), which may be due to vegetable antioxidants. Antioxidants can scavenge free radicals produced in the human body. Free radicals (or reactive oxygen species) can damage tissues and cells. Phenolic compounds account for a major portion of the antioxidant capacity in many plants. Carrots have been ranked 10th in nutritional value among 39 fruits and vegetables (*2*), and research on carrot health benefits continues (*3*).

Carrot is known as an orange vegetable, but other carrot colors exist, some predating orange carrots (*3*). The first carrots were yellow and purple followed by the development of orange carrots in the 15th and 16th centuries. The rapid rise in popularity of orange carrots was not motivated by knowledge of nutrition. Nonetheless, orange carrots resulted in a rich source of provitamin A for consumers. In the past 50 years, carrots have been biofortified by plant breeders so that U.S. carrots today contain >50% more carotenoids than those of 1970 (*4*, *5*), a trend that is unusual among vegetables (*6*).

Pigments in carrots, such as anthocyanins and carotenoids, are antioxidants that can fight disease. Carotenoids are a group of yellow, orange, and red phytochemicals found in most plants. The typical orange carrot contains high amounts of α- and β-carotene, which account for about half of the provitamin A carotenoid found in the food supply. Red carrot color is due to high lycopene content. Yellow carrot color is due to lutein, which might be an important compound in the prevention of macular degeneration (*7*). Purple carrot color is due to anthocyanins. Prepackaged "cut-and-peel", shredded, and other prepared carrots have been introduced into the market, causing consumption to increase in recent years. The consumption of carrots is ranked 6th among 22 commonly consumed vegetables in the United States (*8*).

Traditional breeding and genetic approaches have been used to improve carrot nutrients and color. Biofortified carrots contain increased concentrations of bioactive compounds, such as carotenoids and polyphenols (*9*). Although some research has been done on the carotenoid composition of different colored carrots (*10*), little research has systematically investigated their antioxidant content and antioxidant capacity, especially the relationship between specific antioxidants and total antioxidant capacity. The objective of this research was to compare the antioxidants (e.g., anthocyanins, chlorogenic acid, caffeic acid, and carotenoids) and antioxidant capacity of seven different colored carrots (purple with yellow core, purple with orange core, dark orange, typical orange, yellow, red, and white). Their antioxidant relationships to each other were assessed using the relative antioxidant capacity index (RACI) (*11*).

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MATERIALS AND METHODS

Chemicals. 2,2′-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), chlorogenic acid, caffeic acid, and formic acid (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Lutein, isolated from marigold flowers, was obtained from Kemin Industries (Des Moines, IA). α-Carotene was isolated and purified from high-carotene carrots (*12*). β-Carotene was purified from supplements (GNC Inc., Pittsburgh, PA). Methanol (HPLC grade) was purchased from Fisher Scientific (Springfield, NJ). Cyanidin 3-glucoside chloride was purchased from Chromadex Inc. (Santa Ana, CA). Malvidin-3-galactoside chloride was purchased from Indofine Chemical Co. (Somerville, NJ).

Carrots. Seven carrots, including purple with yellow core, purple with orange core, dark orange, typical orange, yellow, red, and white, were grown by the University of California Desert Research and Extension Station and harvested in March 2008. Genetic stocks used included purple-yellow 309-2, purple-orange B7262, red 410-1, dark orange B2327, orange 320-2, yellow 702-1, and white 211-1 from the USDA carrot breeding program. Carrots were refrigerated at 2 °C and shipped from California to Wisconsin. Upon arrival, carrots were immediately stored at 2 °C.

Extraction and Analysis of Anthocyanins in Carrots. Fresh carrot macerates (0.1−0.5 g) with added internal standard (malvidin-3-galactoside chloride) to determine extraction efficiency were extracted with 10% formic acid in methanol five times (*13*). The extracts were centrifuged and the supernatants combined. Anthocyanins were determined by HPLC.

The Waters HPLC system (Milford, MA) included a 1525 binary pump, 717 autosampler, and 2996 photodiode array detector. An Agilent Zorbax C_{18} (5 μm, 4.6 × 250 mm) with a guard column was used for analysis. Mobile phase A was 10% formic acid in water, and mobile phase B was methanol. B was increased from 0 to 15% in 20 min, followed by an 18 min linear increase to 20%, a 10 min increase to 30%, and a 3 min increase to 55%. The total run time was 53 min. The anthocyanin peaks were identified by comparing their retention times and UV spectra with a former purple carrot extract for which peaks had been identified by MS (*13*). Cyanidin-3-glucoside was used to prepare the standard curve for quantifying the anthocyanins.

Extraction and Analysis of Carotenoids. Carotenoids were determined according to a previous methodology (*10*). Carrot macerate was ground with sodium sulfate, and the internal standard β-apo-8′-carotenal was added to determine extraction efficiency. Alternating washes of dichloromethane and acetone were used. An aliquot of the extract was dried under argon and redissolved in 50:50 (v/v) dichloroethane/methanol before analysis by HPLC (*10*). The separation was performed using a Waters Resolve C_{18} (5 μm 3.9 × 300 mm) column connected to a guard column. Detection was at 450 nm, and carotenoids were quantified using external standard curves of HPLC purified standards. Total carotenoid content was the sum of lutein, lycopene, α-carotene, and β-carotene.

Extraction of the Lipophilic and Hydrophilic Fractions of Carrots. Carrots were freeze-dried and extracted with hexane twice to extract lipophilic antioxidants. Supernatants were pooled, dried under argon, and redissolved in acetone. For the hydrophilic fraction, the residue was extracted with acetone/water/acetic acid (70:29.5:0.5, v/v/v) (*14*).

Total Phenolic Content and Phenolic Acid Composition. Total phenolic content of carrot extract was determined using the Folin−Ciocalteu reagent (*15*). Aliquots of carrot hydrophilic extracts were mixed with diluted Folin−Ciocalteu reagent and then sodium bicarbonate solution after 5 min. After 90 min at room temperature, absorbance of the solution was determined at 750 nm using a UV−visible spectrophotometer. Catechin was used to prepare the standard curve.

Phenolic acids in the hydrophilic extract were analyzed by HPLC using the same conditions as for anthocyanin analysis. Detection was at 330 nm. Chlorogenic acid and caffeic acid were quantified using external standardization.

Determination of Antioxidant Capacity of Carrots by the ABTS and DPPH Methods. Two methods were used to analyze antioxidant capacity as no single method represents antioxidant capacity well (*16*). Antioxidant capacities of both the lipophilic and hydrophilic fractions were determined. Total antioxidant capacity was the sum of antioxidant capacities for the hydrophilic and hydrophobic fractions. For the DPPH method (*17*), aliquots of carrot extract were added to 1 mL of DPPH in ethanol solution, and the absorbance was determined at 515 nm after 30 min at room temperature. The reduction of the absorbance was calculated according to the following equation:

$$\text{inhibition}\% = (\text{Abs}_{t=0} - \text{Abs}_{t=30\text{min}})/\text{Abs}_{t=0} \times 100$$

$\text{Abs}_{t=0\text{min}}$ and $\text{Abs}_{t=30\text{min}}$ were the absorbance of DPPH solution at 0 and 30 min, respectively.

The reduction of the absorbance was plotted against the amount of sample to draw a regression line. The ratio between the sample and Trolox's slope of the regression line was calculated and expressed as the Trolox equivalent antioxidant capacity (TEAC). $ABTS^{\bullet+}$ free radical was produced from the reaction of ABTS and potassium persulfate (*18*). Aliquots of extracts were added to 1 mL of ABTS solution, and the absorbance was determined at 734 nm after 10 min. The inhibition percentage of absorbance and the TEAC were calculated in the same way as for the DPPH method. The RACI, which is an integrated approach to compare antioxidant capacities of different foods or food components measured with two or more chemical assays (*11*), was calculated for total antioxidant capacity, the hydrophilic fraction, and the hydrophobic fraction.

Determination of the Inhibition Capability in Preventing Cholesterol Oxidation during Heating. Hydrophilic and hydrophobic extracts from the same carrot were combined to represent the total antioxidants extracted from the individual carrot. The extracts were combined to mimic the whole vegetable against a control. One milliliter of cholesterol solution (0.1 mg/mL in hexane) and 50 μL of carrot extract were mixed in a glass tube by vortex. The control contained no carrot extract. The sample was dried under argon and heated at 175 °C for 20 min. After the tube cooled, 1 mL of methanol was added to the tube and mixed by vortex. The solution was analyzed by the same HPLC under conditions as for carotenoid analysis, but the mobile phase was acetone/methanol (10:90) at a flow rate of 0.8 mL/min. Detection was set at 215 nm. The percentage of remaining cholesterol was calculated by the following equation: (remaining cholesterol amount/original cholesterol amount) × 100.

Statistical Analysis. All analyses were performed using two replicates from four carrots. Data were expressed as means ± SD. Analysis of variance (ANOVA) and multiple comparisons with Student−Newman−Keuls test were used to investigate differences among samples. $\alpha = 0.05$ was set as the significant criterion. Pearson correlation was used to address the relationship between different parameters. Statistical analyses were performed using SAS (version 9.1, Cary, NC; 2003).

RESULTS AND DISCUSSION

Anthocyanin Content of Carrots. Anthocyanins were detected in only purple-yellow and purple-orange carrots (**Table 1**). Five anthocyanins were identified by comparing the HPLC profiles to published work (*13*, *19*, *20*). These anthocyanins were Cy-3-(2″-xylose-6-glucose-galactoside) (Cy3XGG), Cy-3-(2″-xylose-galactoside) (Cy3XG), Cy-3-(2″-xylose-6″-sinapoyl-glucose-galactoside) (Cy3XSGG), Cy-3-(2″-xylose-6″-feruloyl-glucose-galactoside (Cy3XFGG), and Cy-3-(2″-xylose-6″-(4-coumuroyl)glucose-galactoside) (Cy3XCGG). The total anthocyanin content, which was the sum of the five anthocyanins, was not different between purple-yellow and purple-orange carrots. However, Cy3XSGG content in purple-orange carrot was higher than that in purple-yellow carrot, and Cy3XG, Cy3XFGG, and Cy3XCGG contents in

purple-yellow carrot were higher than those in purple-orange carrot. Among the five identified anthocyanins, Cy3XFGG and Cy3XG were highest in the purple-orange carrot and Cy3XSGG was highest in the purple-yellow carrot (**Table 1**; $P < 0.05$). These data agreed with previous results (*9*, *13*, *19*, *20*).

Phenolic Acid Content and Total Phenolic Content. Several phenolic acids have been reported in carrots (*20*, *21*), but only chlorogenic acid and caffeic acid were quantified here due to standard availability. Purple-yellow carrot contained the highest amount of chlorogenic acid, followed by purple-orange carrot, and it was much higher than that in other carrot colors (**Table 2**). Chlorogenic acid in purple-yellow carrot was 2.5, 13.9, 29.8, 16.3, 61.5, and 19.2 times that in purple-orange, red, dark orange, typical orange, yellow, and white carrots, respectively. The rank of caffeic acid content in carrots was the same as for chlorogenic acid. Caffeic acid content was much less than chlorogenic acid in all carrots; for example, chlorogenic acid content was 52 times that of caffeic acid content in purple-yellow carrots, and the ratio ranged from 33 to 258 across all colors. The predominant phenolic acids in carrots are chlorogenic acid, caffeic acid, *p*-OH-benzoic acid, ferulic acid, and other cinnamic acid isomers (*21*). Chlorogenic acid had the highest content among all phenolic acids (*21*), and in this study it was in the same range as in previous papers. Chlorogenic acid represented 52.4, 57.1, 51.4, and 72.5% of the total phenolic compounds in orange, yellow, white, and purple carrots, respectively. For total phenolic content, which is an important indicator of antioxidant content in food, purple-yellow carrot had the highest value, followed by purple-orange carrot, whereas the other carrots did not significantly differ (**Table 2**), which agrees with a previous study (*22*).

Carotenoid Content. Total carotenoid content, which was the sum of lutein, lycopene, α-carotene, and β-carotene contents, was the highest in dark orange carrot as expected, followed by typical orange, red, purple-orange, and purple-yellow carrot. White and yellow carrots contained only small amounts of carotenoids (**Table 3**). α- and β-carotene were highest in the dark orange carrot, followed by orange carrot, whereas other carrots contained much less. Lutein content was higher in purple-yellow carrot than in all other carrots except yellow carrot. Lycopene in the red carrot was much higher than that in other carrots, as expected. The most abundant carotenoid detected among those analyzed was β-carotene in purple-yellow, purple-orange, yellow, dark orange, and typical orange carrots. The most abundant carotenoids in red and white carrot were lycopene and lutein, respectively. The carotenoid content of the carrots in this research was similar to those reported (*9*, *10*). α- and β-carotene accounted for 13−40 and 44−79% of total carotenoids in typical orange carrots, respectively.

Antioxidant Capacity. Total antioxidant capacity, which was the sum of antioxidant capacities for the hydrophilic and hydrophobic fractions, correlated significantly between the DPPH and ABTS methods (equation $Y = 0.4617X + 0.455$; Y = antioxidant capacity as determined by DPPH method; X = antioxidant capacity as determined by ABTS method; $r = 0.999$) (**Figure 1**). For total antioxidant capacity, both the DPPH and ABTS methods showed that purple-yellow carrot had the highest antioxidant capacity, followed by purple-orange carrot, whereas the antioxidant capacities of the other carrots did not

Table 1. Anthocyanin Content of Purple-Yellow and Purple-Orange Carrots[a]

	μmol/g of dry wt	
	purple-yellow carrot	purple-orange carrot
Cy3XGG[2]	0.66 ± 0.37 bA	1.20 ± 0.82 bA
Cy3XG	6.93 ± 4.94 aA	2.62 ± 2.74 bB
Cy3XSGG	0.61 ± 0.31 bB	9.82 ± 3.22 aA
Cy3XFGG	7.81 ± 4.20 aA	3.85 ± 2.46 bB
Cy3XCGG	1.31 ± 0.83 bA	0.35 ± 0.40 bB
total anthocyanin content	17.3 ± 8.09	17.9 ± 8.60

[a] n = 4 carrots/color analyzed in duplicate. Data with significant differences in the same column are indicated as a, b; data with significant differences in the same row are indicated as A, B. [2] Abbreviations used: Cy3XGG, Cy-3-(2′′-xylose-6-glucose-galactoside); Cy3XG, Cy-3-(2′′-xylose-galactoside); Cy3XSGG, Cy-3-(2′′-xylose-6′′-sinapoyl-glucose-galactoside); Cy3XFGG, Cy-3-(2′′-xylose-6′′-feruloyl-glucose-galactoside; and Cy3XCGG, Cy-3-(2′′-xylose-6′′-(4-coumuroyl) glucose-galactoside).

Table 2. Phenolic Acid Content and Total Phenolic Content of Carrots[a]

	chlorogenic acid (μg/g of dry wt)	caffeic acid (μg/g of dry wt)	total phenolic content (mg of gallic acid equiv/g of dry wt)
purple-yellow	18790 ± 38 a	358.5 ± 38.15 a	38.69 ± 5.37 a
purple-orange	7661 ± 4.9 b	230.7 ± 4.87 b	15.04 ± 1.14 b
red	1347 ± 2.3 c	9.90 ± 2.34 c	2.27 ± 0.10 c
dark orange	630.9 ± 0.56 c	3.25 ± 0.56 c	1.66 ± 0.11 c
orange	1150. ± 0.30 c	4.45 ± 0.30 c	2.34 ± 0.05 c
yellow	305.6 ± 1.59 c	3.16 ± 1.59 c	1.79 ± 0.58 c
white	977.6 ± 2.78 c	11.7 ± 2.78 c	2.35 ± 0.32 c

[a] n = 4 carrots/color analyzed in duplicate. Data with significant differences in the same column are indicated as a−c.

Table 3. Carotenoid Concentrations in Carrots of Various Colors[a]

	μg/g dry of wt				
	lutein	lycopene	α-carotene	β-carotene	total carotenoids
purple-yellow	26.6 ± 4.3 aB	3.68 ± 2.03 bC	18.9 ± 1.90 cB	127.9 ± 16.8 dA	771.0 ± 22.0 e
purple-orange	9.04 ± 0.33 cdC	2.02 ± 0.28 bD	83.7 ± 3.28 cB	239.5 ± 8.04 cA	334.2 ± 11.6 d
red	1.68 ± 1.03 dC	419.4 ± 48.6 aA	1.74 ± 0.69 cC	187.2 ± 18.3 cB	610.1 ± 40.5 c
dark orange	5.53 ± 0.28 dC	7.76 ± 0.89 bC	381.9 ± 18.0 aB	939.7 ± 54.5 aA	1334.7 ± 70.8 a
orange	3.61 ± 1.94 dC	5.09 ± 1.52 bC	228.3 ± 140.8 bB	579.3 ± 79.5 bA	816.3 ± 206.9 b
yellow	19.8 ± 7.88 abB	0.32 ± 0.10 bC	1.86 ± 1.24 cC	30.1 ± 8.1 eA	52.0 ± 16.6 f
white	14.2 ± 8.84 bcA	0.35[b]	0.46 ± 0.33 cB	2.81 ± 3.64 eB	17.6 ± 11.2 f

[a] n = 4 carrots/color analyzed in duplicate. Data with significant differences in the same column are indicated as a−f; data with significant differences in the same row are indicated as A−D. [b] Lycopene was detected in one of four white carrots.

significantly differ (**Table 4**). Hydrophilic extracts had much higher antioxidant capacity than hydrophobic extracts for all carrots. The antioxidant capacity of the hydrophilic extract of purple-yellow carrot was >90 times higher than that of the hydrophobic extract as determined by the ABTS and DPPH methods.

Although the antioxidant capacity of the hydrophobic fraction was much lower than the hydrophilic extract, lipophilic compounds have different functions and/or sites of action in vivo due to different physicochemical properties from hydrophilic compounds (*23*). For the antioxidant capacity of the hydrophilic extract, purple carrot was the highest as determined by both the ABTS and DPPH methods. For the hydrophobic extract as determined by the ABTS method, the antioxidant capacities of orange, dark orange, and red carrots did not significantly differ and were higher than the antioxidant capacities of purple-yellow and purple-orange carrots, whereas yellow and white carrots had the lowest antioxidant capacity. The results of the DPPH method agreed with the ABTS method except that purple-yellow carrot did not significantly differ in antioxidant capacity with orange, dark orange, and red carrots. Research is limited on the antioxidant capacity of carrots, especially different colored carrots. A previous study using the ABTS method showed that the hydrophilic extract of purple-orange carrot had higher antioxidant capacity than typical orange, white, red, and yellow carrots, whereas the hydrophobic extract of purple-orange and orange carrots had higher antioxidant capacity than white, yellow, and red carrots (*24*). The results of antioxidant capacity of purple-orange and orange carrots in this study were in the same range as data in the previous paper (*9*).

Multiple reaction mechanisms and different phase locations are usually involved in measuring the antioxidant capacity of a complex food system. Therefore, a simple universal method by which "total antioxidant capacity" can be measured accurately and quantitatively does not exist. Different methods have been used to measure the antioxidant capacity of food. A new concept was developed called RACI to integrate results from multiple methods. RACI was proposed from the perspective of statistics by integrating food antioxidant capacity data determined by several methods to provide a reasonably accurate rank of antioxidant capacity among different foods (*11*). RACI in this research was calculated from the ABTS and DPPH methods to compare antioxidant capacities among the carrot types. RACI showed that purple-yellow carrot was the highest, followed by purple-orange carrot, whereas the other carrots had only slight differences. RACI for the hydrophilic antioxidant capacity gave a similar rank of carrot antioxidant capacity compared to RACI for total antioxidant capacity. The RACI value for hydrophobic extracts from high to low was dark orange, red, orange, purple-yellow, purple-orange, yellow, and white.

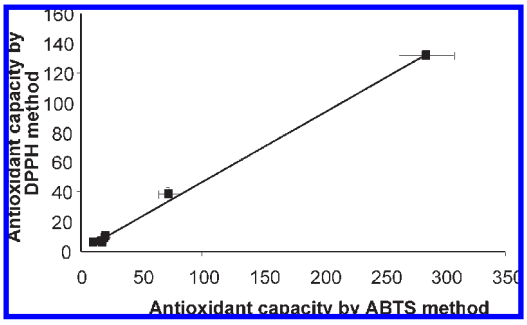

Figure 1. Correlation of the antioxidant capacity (μmol of Trolox equiv/g) of carrots as determined by the ABTS and DPPH methods (r = 0.999, P < 0.0001).

Oxidation of Cholesterol during Heating. Meat, eggs, and many processed foods contain cholesterol, which is easily oxidized during heating. The oxidation products produced are harmful to human health (*25*). The ability of carrot antioxidants to prevent the oxidation of cholesterol during heating was studied. The remaining cholesterol with added carrot extracts, except extracts from yellow and white carrots, were all significantly higher than the control (**Figure 2**), indicating that the antioxidants of these carrots can prevent the oxidation of cholesterol during heating. Purple-yellow carrot had the highest ability to prevent the oxidation of cholesterol during heating.

Correlation of Antioxidants and Antioxidant Capacity. Chlorogenic acid, total phenolics, total anthocyanins, Cy3XG, Cy3XFGG, and Cy3XCGG contents correlated significantly with RACI of total antioxidant capacity, as well as RACI of the hydrophilic fraction of carrots (**Table 5**). This correlation showed that (i) chlorogenic acid was a major antioxidant in different colored carrots; (ii) anthocyanins, especially Cy3XG, Cy3XFGG, and Cy3XCGG, contributed significantly to the antioxidant capacity of purple-yellow and purple-orange carrots; and (iii) major antioxidants in carrots were phenolics (phenolic acid and flavonoids). Anthocyanins are flavonoids, and chlorogenic and caffeic acids are phenolic acids. Phenolics were mainly in the hydrophilic extract, and the antioxidants in the hydrophobic fraction were mainly carotenoids and tocopherols. Total carotenoid content or individual carotenoids did not correlate with the RACI of total antioxidant capacity, which showed that carotenoids were not major antioxidants of the different colored carrots. However, the carotenoids correlated significantly with the RACI of the hydrophobic extract (P = 0.02), showing that the carotenoids were major antioxidants in the hydrophobic extract. RACI of the hydrophilic extract had a very positive correlation with RACI of the total extract, whereas RACI of the hydrophobic extract had no relationship with it

Table 4. Antioxidant Capacity of Hydrophilic and Hydrophobic Extracts and Total Antioxidant Capacity of Carrots As Determined by the ABTS and DPPH Methods[a]

	μmol of Trolox equiv/g of dry wt					
	ABTS			DPPH		
	hydrophilic	hydrophobic	total	hydrophilic	hydrophobic	total
purple-yellow	282.7 ± 23.4 a	2.24 ± 0.19 b	285.0 ± 23.5 a	129.7 ± 7.45 a	1.37 ± 0.22 ab	131.1 ± 7.62 a
purple-orange	71.2 ± 8.24 b	1.83 ± 0.49 b	73.1 ± 8.43 b	37.67 ± 4.15 b	1.06 ± 0.08 b	38.7 ± 4.09 b
red	16.2 ± 3.48 c	3.48 ± 0.70 a	19.7 ± 3.87 c	7.17 ± 0.32 c	1.73 ± 0.21 a	8.91 ± 0.27 c
dark orange	12.6 ± 2.18 c	4.00 ± 0.14 a	16.6 ± 2.07 c	4.77 ± 0.65 c	1.66 ± 0.29 a	6.43 ± 0.39 c
orange	16.5 ± 0.54 c	3.62 ± 1.03 a	20.2 ± 1.50 c	8.60 ± 0.84 c	1.52 ± 0.17 a	10.1 ± 0.92 c
yellow	9.51 ± 2.10 c	0.59 ± 0.18 c	10.1 ± 2.26 c	5.64 ± 0.31 c	0.20 ± 0.08 c	5.84 ± 0.25 c
white	17.3 ± 2.85 c	0.33 ± 0.07 c	17.6 ± 2.88 c	6.01 ± 0.41 c	0.20 ± 0.03 c	6.21 ± 0.44 c

[a] n = 4 carrots/color analyzed in duplicate. Data with significant differences in the same column are indicated as a−c.

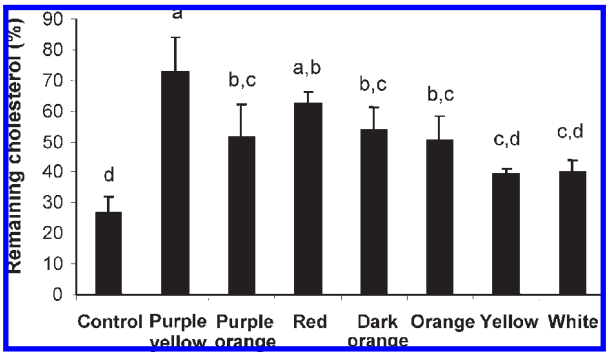

Figure 2. Percentage of remaining cholesterol after heating with added carrot extract. Data with significant differences are labeled with different letters.

Table 5. Correlation Coefficients between Specific Antioxidants in Carrots and Antioxidant Capacity Expressed as the Relative Antioxidant Capacity Index (RACI)

	RACI		
	hydrophilic	hydrophobic	total
total phenolic content	0.987	NS[a]	0.992
total carotenoid content	NS	0.835	NS
total anthocyanin content	0.772	NS	0.791
Cy3XG[b]	0.983	NS	0.989
Cy3XFGG	0.958	NS	0.967
Cy3XCGG	0.997	NS	0.999
chlorogenic acid	0.915	NS	0.932
cholesterol	NS	NS	0.756

[a] NS means that the correlation was not significant at $P < 0.05$. [b] Abbreviations used: Cy3XG, Cy-3-(2″-xylose-galactoside); Cy3XFGG, Cy-3-(2″-xylose-6″-feruloyl-glucose-galactoside; and Cy3XCGG, Cy-3-(2″-xylose-6″-(4-coumuroyl) glucose-galactoside).

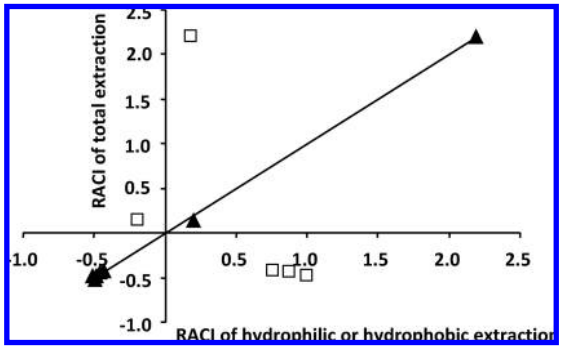

Figure 3. Relationship between the relative antioxidant capacity index (RACI) of total antioxidant capacity and RACI of hydrophilic (▲) or hydrophobic (□) extracts of different colored carrots. The regression line for the hydrophilic extract was drawn in the figure and its equation was as follows: RACI of total extraction = 1.00 × RACI of hydrophilic extract, $r^2 = 0.999$. RACI of total antioxidant capacity and RACI of the hydrophobic extract were not correlated.

(**Figure 3**). This further demonstrated that the contribution of the hydrophilic extract to total antioxidant capacity was highly significant. The results of the cholesterol method correlated with the RACI value of the total extract ($P = 0.050$), but did not correlate with total phenolic content, total carotenoid content, total anthocyanins, or the RACI of hydrophilic and hydrophobic extracts (**Table 5**).

Carrot Colors. The carrots evaluated in this study include single genotypes from one location of each color or color combination, for practical reasons. Carrots were harvested from the field in California and shipped to Wisconsin. Four carrots from each color group were selected for analysis. The SDs presented in this study likely represent the variation among carrots within a single season. Higher SDs would be expected between seasons (*26*). Whereas purple carrot color always indicates the presence of anthocyanins, orange indicates the presence of α- and β-carotene, yellow the presence of lutein, and red the presence of lycopene; the quantities of each of these pigments varies widely across all color classes. Consequently, the results presented are representative but may not necessarily be extrapolated to other carrots of the same color. Carotenoid content may be affected by genotype, maturity, growing season, and growing conditions (*4*, *5*, *10*, *20*, *21*). Different parts of the carrot also contain different amounts of carotenoids; for example, early orange carrot cultivars often had yellow xylem (cores), and the outer phloem (cortex) of orange carrots can contain twice the amount of β-carotene as the inner phloem (*4*). Therefore, sample genotype, preparation, and sampling procedures may affect the results, which are also true for the detection of anthocyanins, phenolic acids, and antioxidant capacity in carrots.

These results show that purple carrots, such as purple-yellow or purple-orange, had high antioxidant capacity and antioxidant content. Another class of phytochemicals isolated from an aqueous extract of purple carrots, that is, polyacetylenes, decreased expression of inflammatory proteins in macrophage and endothelial cells (*27*). Thus, it may be more beneficial for human health to eat purple carrots compared to other colors of carrots on the basis of antioxidant capacity and biological effects.

The absorption and metabolism of carrot antioxidants are important factors that affect their function in vivo. In vitro methods may not reflect in vivo antioxidant effects. Some research with carrots of various colors has shown promising results in vivo. For example, both lycopene and β-carotene were bioavailable from red carrots in humans (*26*) and animals (*28*); anthocyanins from purple carrots have been found to be bioavailable and can be absorbed intact (*3*, *13*); gerbils fed purple-orange-red carrot had higher antioxidant capacity in liver compared to control (*9*). Finally, although lutein content is currently low in yellow carrots compared with β-carotene in typical orange carrots, it is highly bioavailable in humans (*29*). The bioavailability of the antioxidants in carrots, especially the purple varieties, needs to be further investigated in humans. Carrot varieties that are high in β-carotene, lutein, lycopene, and anthocyanins have been developed and could contribute to total antioxidant intake. The consumption of these carrots can provide not only vitamin A but also other functional compounds as shown in human bioavailability studies (*13*, *26*, *29*). This study provides useful information for carrot growers targeting new consumer markets and product development, for carrot breeders developing more nutritious carrots, and for consumers as a guideline to make healthy choices. Indeed, although orange carrot was a preferred choice in a consumer evaluation of sensory characteristics, purple was also well-liked (*10*). Therefore, breeding purple, red, and yellow carrots for improved crispness and sweetness may result in broader acceptance.

SAFETY

Use of a fume hood for volatile organic solvents is recommended.

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