DOI:10.1021/jf9022405

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Influence of Pre- and Postharvest Factors on β-Carotene Content, Its in Vitro Bioaccessibility, and Antioxidant Capacity in Melons

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Fresh and stored melons were analyzed for total and bioaccessible β-carotene content, as well as total antioxidant potentials, to investigate the effects of genotype, season, sowing time, and storage on nutritional quality. There were significant cultivar effects on β-carotene content ($P < 0.001$), ranging from 1 to 23 mg kg^{-1} of fresh weight (FW), its bioaccessibility ($P < 0.005$), ranging from 79 to 94.5%, and for antioxidant potential ($P < 0.001$), ranging from 1569 to 2267 μmol of Fe^{2+} kg^{-1} of FW of melons. Sowing time significantly affected β-carotene contents ($P < 0.017$) and antioxidant potentials ($P < 0.001$), but these were highly cultivar dependent, with season having no effect. Postharvest storage over 4 weeks at 7 °C resulted in losses of antioxidant potential and β-carotene content independent of cultivar. Cultivar and postharvest management were major determinants of nutritional quality of the melons evaluated.

KEYWORDS: Melon (*Cucumis melo*); β-carotene; ferric reducing antioxidant potential (FRAP); oxygen radical absorbance capacity (ORAC); bioaccessibility; preharvest; postharvest

INTRODUCTION

Epidemiological studies indicate that the intake of adequate amounts of fruits and vegetables is important for human health and well-being. Diets rich in fruits and vegetables are associated with a reduced risk for coronary heart disease and possibly other major chronic diseases (*1*, *2*). Awareness of these benefits, and heightened individual responsibility for health, has resulted in consumers making more informed choices about food purchases. Now, more than ever, there is an increased demand for “natural” foods that deliver health benefits, but do not compromise on appearance and organoleptic properties. Pre- and postharvest factors can contribute to meeting this demand for “natural,” nutritious plant foods (*3*−*5*). Preharvest factors include genotype, environment, and horticultural management, whereas handling, storage (length of time and conditions), and processing are examples of postharvest factors. Understanding how these factors affect the nutritional quality of fruits and vegetables will help meet consumers’ demands.

This study focused on two widely consumed melon types, *Cucumis melo*, var. *reticulatus* and *C. melo*, var. *inodorus*, of which there are numerous cultivars grown in the world’s warm dry regions. In 2005, production worldwide was approximately 28 million tonnes with a market value exceeding U.S. $1 billion (*6*). Cultivar is an important preharvest factor determining the nutritional quality and consumer appeal of fruits and vegetables (*3*−*5*, *7*−*9*). Melons are polymorphic (*10*) and provide ideal material to investigate cultivar effects on the nutritional quality of fruit. The sweet, edible fruits differ greatly between cultivars in size, shape, surface texture, flesh color, cavity size, flavor, aroma, firmness, and weight. There is also evidence that cultivar, environment, and management variables affect levels of micronutrients and phytochemicals in melons (*5*, *8*, *10*). Orange-fleshed melons are known to have relatively high levels of β-carotene, a potent antioxidant and a precursor to vitamin A (*11*). Fruits and vegetables rich in β-carotene are significant sources of vitamin A for much of the world’s population (*12*). Obviously, for maximum benefit, the carotenoid must be not only present at high concentrations, but also readily bioaccessible (i.e., a high proportion efficiently incoporated into micelles during digestion in the small intestine). However, few cultivars have been characterized nutritionally under changing environmental conditions, and even fewer have been characterized for variation in nutrient bioaccessibility, which remains largely unexplored. Using an in vitro digestion model, it has recently been demonstrated that bioaccessibility of β-carotene is directly related to provitamin A content in different *Manihot esculanta* (cassava) cultivars (*13*). Genetic variation in yield and adaptation to biotic and abiotic stress have

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Published on Web 01/12/2010
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Table 1. Melon (*Cucumis melo*) Cultivars[a]

variety	cultivar	suture	net	flesh color	harvest method	shape (*l*/*w*)	weight (g)
reticulatus	Eastern Star*	+	+	orange	slip	1.1 ± 0.01	1680 ± 50
reticulatus	Northern Sky*	+	+	orange	slip	1.2 ± 0.01	2357 ± 87
reticulatus	Frontier	−	+	orange	slip	1.2 ± 0.02	1678 ± 74
reticulatus	Hot Shot	−	+	orange	slip	1.2 ± 0.01	1859 ± 89
reticulatus	Dubloon*	−	+	orange	slip	1.3 ± 0.01	1380 ± 37
reticulatus	Southern Cross*	+	+	orange	slip	1.2 ± 0.01	1591 ± 58
reticulatus	Chantele*	+	+	orange	clip	1.3 ± 0.02	1619 ± 56
inodorus	Solid Gold	−	−	white	clip	1.2 ± 0.01	1814 ± 69
reticulatus	CSIRO F1 hybrid*	−	+	orange	clip	1.0 ± 0.01	1120 ± 30
reticulatus	Delicious	−	+	green	clip	1.0 ± 0.02	670 ± 50

[a] All 10 cultivars were sown the first season (all three sowings) (see **Table 2** for sowing dates). * indicates the six cultivars sown in the second season. Suture and net refer to rind characteristics and are longitudinal vein tracts and surface netting, respectively. Melons were harvested according to whether they formed an abscission zone (slip) or required cutting from the vine (clip). Shape = length divided by width (1 = spherical). Numerical data are means (n = 100) ± mean standard error of difference ($P < 0.05$).

Table 2. Sowing and Harvest Dates of Melon (*C. melo*) Cultivars[a]

season	sowing	sowing date	harvest dates	av temperature[b] (°C)	av solar radiation[b] (MJ m^{-2})[c]
1	1	Nov 19, 2004	Feb 2−21, 2005	max 28.7 ± 1.2 min 13.9 ± 0.6	25.4 ± 1.2
	2	Dec 10, 2004	Feb 23−March 21, 2005	max 30.3 ± 1.0 min 13.3 ± 0.8	28.0 ± 0.6
	3	Dec 31, 2004	March 20−April 7, 2005	max 27.8 ± 0.9 min 12.8 ± 0.9	19.2 ± 1.0
2	1	Dec 12, 2005	Feb 28−March 16, 2006	max 30.6 ± 1.7 min 16.7 ± 1.3	NA[d]

[a] Seasons 1 and 2 are 2004/2005 and 2005/2006, respectively. Temperature and solar radiation data in the weeks leading up to harvest were provided by the Bureau of Meteorology, Commonwealth of Australia (http://www.bom.gov.au). [b] Values during harvest periods: max, maximum; min, minimum. [c] MJ m^{-2}, megajoules per square meter. [d] NA, not available.

been investigated rigorously (see *14* and references cited therein). The more recent focus on the production of biofortified crops to improve human health has, despite the technical difficulty and expense, resulted in the screening of cultivars for variation in nutritional quality (*15*−*17*). For example, an investigation driven by the linkage between lycopene intake and reduced prostate cancer risk has shown that the carotenoid profiles of tomatoes vary significantly among cultivars (*18*).

The objective of this study was to collect data over multiple sowing dates and seasons to identify melon cultivars with superior nutritional quality. Accordingly, the nutritional quality of 10 cultivars, producing orange-, green-, and white-fleshed melons, was studied. Nutritional quality was determined by measuring β-carotene contents and antioxidant potentials. An in vitro digestion system was used to estimate the bioaccessibility of β-carotene for the cultivars producing melons with the highest carotenoid content.

MATERIALS AND METHODS

Plant Material. Nine commercial F1 cultivars (Syngenta, Australia) and one F1 hybrid selection from CSIRO's melon breeding program were assessed in 2004/2005 (**Table 1**). Six cultivars with the highest nutritional quality were evaluated again in 2005/2006.

Trial Design. The trials were established at Merbein, northwestern Victoria, Australia (142° 2′ E; 34° 12′ S), in a Coomealla sandy loam soil (*19*). Seeds were sown directly into a bed cultivated to a fine tilth approximately 30 cm deep to which a fertilizer mix (N:P:K 5%:6%:6%; Hydrocomplex, Yarra, Aust. Pty. Ltd.) was incorporated at a rate of 1200 kg ha^{-1}. The seed bed was overlain with black polythene mulch to control weeds and irrigated via a dripper line placed under the mulch. There were four replicate plots per cultivar, per sowing. Each replicate plot was 3.5 m in length, and vine spacing was 0.5 m within plots.

There were three sequential sowing dates in 2004/2005 and just one in 2005/2006 (**Table 2**). Maximum and minimum temperatures and solar radiation the weeks leading up to harvest were provided by the Bureau of Meteorology, Commonwealth of Australia (http://www.bom.gov.au) (**Table 2**). Melons were harvested in accordance with commercial practice; cultivars that formed an abscission zone where the melon is attached to the pedicel (slip) (*20*) were harvested at 3/4 slip; nonslip cultivars were harvested by clipping the peduncle when rind color changed (sometimes subtly) and the leaf closest to the fruit senesced (clip) (**Table 1**). Melons were harvested in the morning and immediately placed in pairs within lidless cardboard boxes (30 × 40 cm) on wire mesh shelving in a cool room (7 °C) to remove field heat.

Storage. Harvested melons (n = 6−8) were stored in a cool room at 7 °C and removed at 0, 2, and 4 weeks for analysis. Multiple temperature loggers (Thermocrons and eTemperature tracking software, OnSolution Pty. Ltd., Aust.) were placed randomly throughout the cool room to continuously monitor positional variations in temperature during melon storage.

Color Measurements. Flesh color for a sample of 9−10 fresh melons was measured in both seasons immediately after harvest using a Minolta Chroma Meter (CR-300 series, Minolta Camera Co. Ltd., Osaka, Japan). Fruits were dissected longitudinally, and color was measured at five locations 1.5 cm below the epidermis across the cut surfaces and averaged for each fruit. The chroma meter was calibrated using a white color standard, and color was expressed in the tristimulus L^* (lightness), a^* (green to red), and b^* (yellow to blue), from which hue angle and chroma (*21*, *22*) were calculated.

β-Carotene Determination. Mesocarp of each sample (n = 5) was extracted in duplicate under restricted lighting until colorless with tetrahydrofuran (THF) based on the methods of Bushway (*23*) and Hegazi et al. (*24*) and adapted for use in our laboratory. Ascorbic acid (10 mL of a 1% w/v solution) was added to a 5 g sample and then homogenized using a high-speed blender for 1 min. Ten milliliters of 9:1 v/v methanol/THF was added, the sample was homogenized for a further 1 min and centrifuged at 2000*g* for 10 min at 4 °C, and the supernatant was transferred to a 100 mL volumetric flask. The extraction procedure was repeated with 10 mL volumes of THF until colorless. The combined supernatants were made to volume (100 mL) with THF. Aliquots (2 mL) were back-extracted with an equal volume of hexane following the addition of 200 μL of 10% w/v aqueous NaCl. The hexane was evaporated to dryness under a stream of

N_2 and the dried extract stored at −20 °C. Samples were reconstituted with mobile phase immediately prior to analysis by high-performance liquid chromatograph (HPLC). Carotenoids were separated on a Microsorb-MV 100-5 μm C18 column (250 mm × 4.6 mm; Varian) using a HPLC (LC1150, GBC Scientific Aust.; SCL 10A, Shimadzu Corp., Kyoto, Japan) equipped with a photodiode array detector (LC5100, GBC; SPD-M10Avp, Shimadzu) and refrigerated sample compartment (LC1650, GBC; SIL 10A, Shimadzu). The mobile phase consisted of acetonitrile (55%), methanol (22%), dichloromethane (11.5%), and hexane (11.5%). Ammonium acetate (0.02%) was added to the mobile phase to achieve proton equilibrium to diminish the peak tailing and mask silanol-free groups (*24*). Flow rate was 1 mL min^{-1}. β-Carotene was detected at 450 nm and identification achieved by comparison of sample retention times and visible spectra with those of standards. Known amounts of lutein, α- and β-carotene, and lycopene (BDH, Poole, U.K.) were used as authentic standard pigments for calibration. Canthaxanthine (Dr. Ehrenstorfer GmbH, Germany) was used as an internal standard. Carotenoid content was expressed as milligrams per kilogram of fresh weight (FW).

β-Carotene in Vitro Bioaccessibility Assays. Only fruits from the second season were assessed for bioavailability of β-carotene. Samples (n = 3) were subjected to simulated human gastric and pancreatic digestion based on the method of Miller et al. (*25*), with modifications to optimize carotenoid extraction (*26*). Two hundred microliters of 150 mM ascorbic acid and 9.8 mL of 0.9% saline solution were added to 1 g of finely chopped mesocarp of each sample, and the pH was adjusted to 2.0 with 5 M HCl. One milliliter of pepsin solution (20 mg mL^{-1} in 0.1 M HCl) was then added, and the sample tubes were filled with N_2 and incubated at 37 °C for 60 min with gentle shaking. The pH was then adjusted to 5.0 with 1 M $NaHCO_3$, and 3 mL of bile/pancreatin solution (300 mg of bile and 50 mg of pancreatin in 35 mL of 0.1 M $NaHCO_3$) was added. The pH was adjusted to 7.0, and the sample tubes were filled with nitrogen and incubated as before for 2 h. A 15 mL subsample of the final digest was centrifuged at 2000g for 10 min. Aliquots (2 mL) of the supernatant were then back extracted into hexane and evaporated to dryness under nitrogen. The residue was dissolved in mobile phase, as described previously, for analysis by HPLC. Carotenoid bioaccessibility was expressed as a proportion (%) of the amount of carotenoid present in the original undigested sample.

Antioxidant Potential Assays: Sample Preparation. Fresh melons were analyzed on the same day as or the day after harvest. Mesocarp tissue was blended with a hand-held domestic food homogenizer (Bamix, Switzerland) to a uniform consistency, and subsamples were stored at −80 °C for later analysis. Samples (n = 12) were thawed prior to analyses, and duplicate 5 g samples were homogenized with 15 mL of high-purity water (Milli-Q plus, Millipore) using an Ultra Turrax disperser (T25 Basic IKA, WERKE, Germany) for 1 min. High-purity water was used to eliminate or minimize any metal ion contamination, which can introduce errors into the FRAP assay. The homogenates were then centrifuged (2000g at 4 °C for 10 min), and the clear supernatant was retained for further analyses.

Ferric Reducing Antioxidant Power (FRAP). FRAP was assessed in both seasons. The FRAP value was determined according to the method of Benzie and Strain (*27*) using a Cobas Bio centrifugal analyzer (Roche Diagnostics Systems, Branchburg, NJ) and a UV−vis 918 spectrophotometer (GBC Scientific, Melbourne, Australia). A subsample of the supernatant (please refer to the sample preparation section above) were diluted 2 times with high-purity water. Ten microliters of sample was added to 300 μL of FRAP reagent (25 mL of 300 mmol of acetate buffer L^{-1}, 2.5 mL of 10 mmol 2,4,6-tripyridyl-1,3,5-triazine L^{-1} in 40 mmol HCl L^{-1}, and 2.5 mL of 20 mmol $FeCl_3 \cdot 6H_2O$ L^{-1}) and the absorbance at 593 nm recorded after a 4 min incubation at 37 °C. The FRAP value was obtained by comparing the change in absorbance of the test mixture to that of Fe^{2+} standard solutions. The results were expressed in micromoles of Fe^{2+} equivalents per kilogram of FW.

Oxygen Radical Absorbance Capacity (ORAC). Only melons harvested in the second season were assessed for ORAC. A subsample of the supernatant (as above) was diluted 10 and 20 times with phosphate buffer and the ORAC value of the diluted samples determined according to the method of Huang et al. (*28*) with slight modification. A 96-well, black plate (BMG Labtech Pty. Ltd., Australia) was prepared by the addition of 20 μL of diluted sample, standard, or blank followed by 200 μL of phosphate buffer (75 mM, pH 7.0) containing fluorescein at 0.096 μM. The plate was incubated at 37 °C for 15 min before the addition of the radical-generating 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) solution (20 μL at 79.7 mM in phosphate buffer). The plate was then shaken and monitored using a fluorescence plate reader (Perkin-Elmer, Wallac, Victor^{3}V, Turku, Finland) in the kinetic mode with readings taken every 2 min for at least 90 min. Solutions of Trolox (Sigma-Aldrich), an analogue of vitamin E, at 0, 6.25, 12.5, 25, and 50 μM were used as standards. The area under the curve (AUC) for standards, samples, and blanks was calculated according to the method of Huang et al. (*28*). The net AUC for the samples was then calculated according to the equation

$$\text{netAUCx} = \text{AUCx} - \text{AUCblk}$$

where AUCx is the area under the curve for sample x and AUCblk is the area under the curve for the blank. The net AUC values for the standards were plotted to produce a standard curve from which ORAC values for the samples were calculated. The ORAC values were expressed in micromoles of Trolox per kilogram of FW.

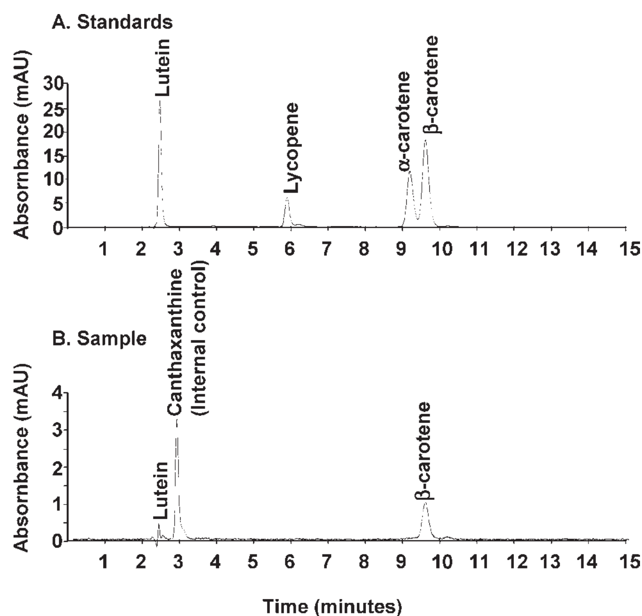

Figure 1. HPLC chromatogram of known standards (**A**) used to calibrate and identify peaks in the spectra of samples (**B**).

Data Analyses. Where the design was balanced, data were subjected to analysis of variance (ANOVA). Where the design was unbalanced, with respect to the number of samples, data were analyzed using restricted maximum likelihood (REML) linear mixed model analysis. Sources of variation (cultivar, sowing, season, and interactions) were considered to be significant if the F ratio or Wald statistic had a probability of ≤0.05. Significant differences between means were identified using the average standard error of difference (sed). Correlations were identified by the Pearson product-moment correlation coefficient (r) with a two-sided test (P < 0.05).

RESULTS

Cultivar. Fruits from the 10 cultivars varied in size, shape, rind characteristics (netting and suturing), and flesh (mesocarp) color (**Table 1**). Orange-fleshed melons are primarily a rich source of β-carotene and hence an important source of provitamin A (*29*, *30*). It was apparent from the HPLC chromatograms (**Figure 1** and data not shown) that β-carotene was the major carotenoid present in the melons, with much smaller amounts of lutein. We have, therefore, reported only β-carotene contents. Cultivar was a significant source of variation for β-carotene (P < 0.001), accounting for approximately 77% of the observed variation in that parameter in the first season (**Figure 2**). Mean β-carotene

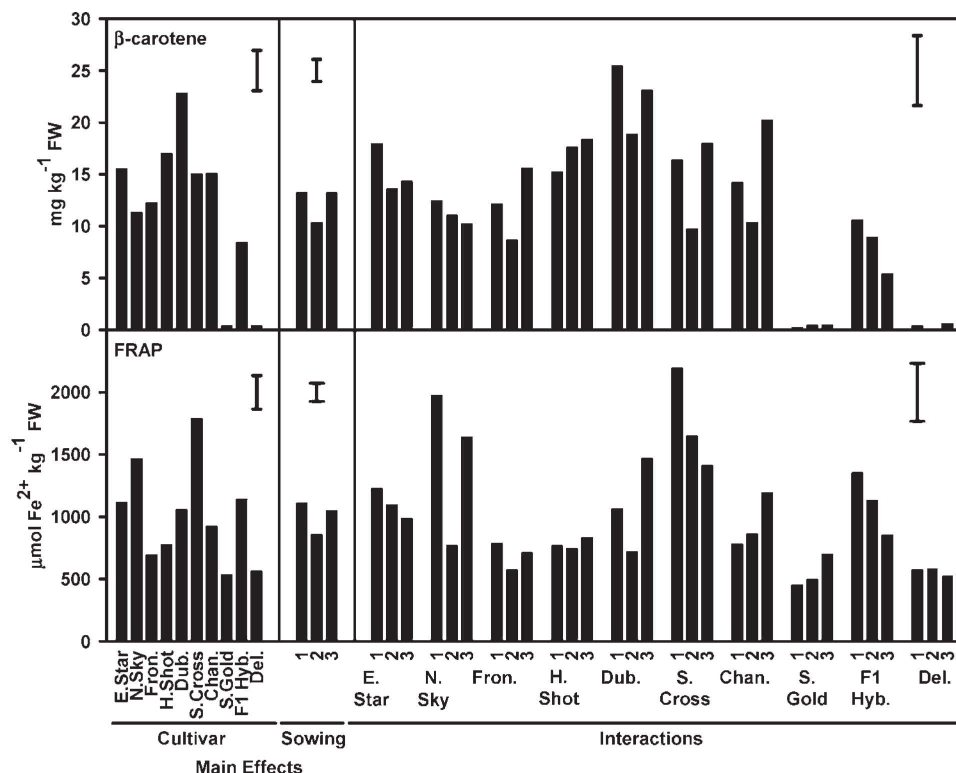

Figure 2. β-Carotene content and ferric reducing antioxidant power (FRAP) of fruit flesh sampled from melon (*C. melo*) cultivars. E. Star, Eastern Star; N. Sky, Northern Sky; Fron., Frontier; H. Shot, Hot Shot; Dub., Dubloon; S. Cross, Southern Cross; Chan., Chantele; S. Gold, Solid Gold; F1 Hyb., F1 hybrid from the CSIRO melon breeding program; Del., Delicious. Data are shown for the three staggered sowings in the first season (see **Table 2** for sowing dates). Mean data (n = 20) for main effects (cultivar and sowing) and interactions are shown. Error bars are mean standard errors of difference.

levels varied from <1 to approximately 23 mg kg^{-1} of FW between cultivars. The highest mean β-carotene concentration was observed in the orange-fleshed Dubloon cultivar (22.83 mg kg^{-1} of FW) followed by the other orange-fleshed cultivars. β-Carotene levels were lowest in melons of the white-fleshed Solid Gold and the green-fleshed Delicious (0.36 mg kg^{-1} of FW) cultivars. This is consistent with published results indicating that the intensity of the orange color in the flesh of different melons is related to their β-carotene content (*31*).

The color of the flesh was measured in fruits of each cultivar (**Table 3**). The melons with orange flesh had higher a^* values as compared to white-fleshed (Solid Gold) and green-fleshed (Delicious) fruits. The flesh hue angle, a^*, b^*, and chroma (**Table 3**) were correlated with β-carotene content (r = −0.930, 0.905, 0.805, and 0.783, respectively; $P < 0.001$, < 0.001, $= 0.007$, and $= 0.005$, respectively), but there was no correlation between L^* (lightness) and β-carotene content.

Postdigestion β-carotene content and percent bioaccessible β-carotene were cultivar dependent (P = 0.002 and 0.005, respectively), accounting for $>70\%$ of the variability in both responses (**Figure 3**). Melons of the CSIRO-bred F1 hybrid had the highest β-carotene bioaccessibility (105%) and those of Chantele the lowest (79.9%). Error in the bioaccessibility assay contributed to $>100\%$ β-carotene bioaccessibility of CSIRO F1 hybrid melons (**Figure 3**). Postdigest, Southern Cross melons had the highest β-carotene content (17.11 mg kg^{-1} of FW) and those of Northern Sky the lowest (10.83 mg kg^{-1} of FW). Pre- and postdigest β-carotene levels were positively correlated (r = 0.914; $P < 0.010$), but there was no significant correlation between percent bioaccessibility and predigest content of β-carotene.

Cultivar was also a significant source of variation in FRAP in the first season ($P < 0.001$), accounting for approximately 35% of the observed variation. FRAP values varied from 534 μmol of Fe^{2+} kg^{-1} of FW in the white-fleshed Solid Gold melons to 1791 μmol of Fe^{2+} kg^{-1} of FW in the orange-fleshed fruit from the Southern Cross cultivar (**Figure 2**). Although FRAP values were generally higher in melons from orange-fleshed cultivars compared to those of white-fleshed cultivars, a^*, b^*, hue angle, and chroma were not correlated with FRAP values. This was due to significant variation in FRAP between the orange-fleshed cultivars: Hot Shot and Frontier fruit had FRAP levels of 778 and 692 μmol of Fe^{2+} kg^{-1} of FW, respectively, whereas the orange-fleshed melons from the CSIRO-bred F1 hybrid had relatively higher FRAP levels (1139 μmol of Fe^{2+} kg^{-1} of FW). However, there was a weak negative correlation between FRAP values and L^* values (r = −0.639; P = 0.047).

FRAP and ORAC values were determined for the orange-fleshed melons from the six cultivars grown in the second season (**Figure 4**), because these methods target different mechanistic aspects of antioxidant activity. The FRAP procedure measures the ability to reduce ferric ions to ferrous ions by antioxidants in hydrophilic extracts and equates this to antioxidant potential. The ORAC method determines the capacity of the sample to inhibit the action of peroxyl radicals and so reflects the physiologically relevant action of chain-breaking antioxidants (*32*). To have a direct comparison of the two assays, the FRAP data in **Figure 4** were obtained in a further assay, which was run concurrently with the ORAC assay. Thus, these data are independent of the data shown in **Figure 1**. The FRAP and ORAC assays showed positive correlation (r = 0.857; $P < 0.010$) in all of the cultivars except Chantele.

Season and Sowing Time. Overall, season had no effect on β-carotene content of the melons from the different cultivars (**Figure 5**). However, a significant season × cultivar interaction indicated that fruits from three of the six cultivars (Northern

Table 3. Minolta Chroma Meter Values Obtained for Fruit Flesh of Melons Harvested from 10 Cultivars Sown over Three Dates in One Season[a]

		cultivar										
	sowing	E. Star	N. Sky	Fron.	H. Shot	Dub.	S. Cross	Chan.	S. Gold	F1 hyb.	Del.	all
*L**	1	55.4 d−g	50.7 n	54.9 e−i	54.1 h−k	52.8 kl	51.2 mn	56.9 bc	54.1 g−k	53.9 jk	55.2 d−i	54.0 c
	2	55.2 e−j	53.1 kl	56.6 b−d	55.4 d−g	55.3 d−h	53.1 k-l	57.6 ab	58.2 a	53.1 kl	55.9 c−e	55.5 a
	3	54.8 f−j	52.0 l−n	53.3 kl	54.8 f−j	54.0 i−k	55.5 d−f	55.3 d−h	57.6 ab	55.4 d−h	52.1 lm	54.6 b
	all	55.1 b	52.0 e	55.0 b	54.8 bc	54.1 c	53.2 d	56.7 a	56.6 a	54.1 c	54.5 bc	
*a**	1	7.1 a	4.2 kl	5.4 f−h	6.5 b	6.5 b	6.1 b−d	6.5 b	−1.4 n	4.1 kl	−8.2 r	3.8 a
	2	5.9 c−e	3.8 l	4.9 ij	6.3 bc	6.4 b	4.9 ij	5.8 d−f	−2.2 o	2.7 m	−6.8 q	3.2 b
	3	5.5 e−g	4.2 kl	5.0 hi	4.5 jk	5.7 d−g	5.3 g−i	6.5 b	−1.1 n	2.6 m	−6.2 p	3.3 b
	all	6.2 a	4.0 e	5.1 d	5.8 b	6.2 a	5.4 c	6.2 a	−1.6 g	3.2 f	−7.1 h	
*b**	1	23.0 a	18.2 m	20.1 h−k	21.6 b−f	20.9 e−i	19.5 kl	22.0 a−c	5.4 q	19.4 kl	18.1 mn	18.4 a
	2	21.7 b−e	20.1 h−k	20.7 f−j	22.0 a−d	20.9 e−i	21.0 d−h	22.4 ab	6.6 p	18.3 m	15.3 o	18.6 a
	3	21.1 c−g	19.1 lm	19.7 j−l	19.9 i−l	20.3 g−k	21.3 c−f	22.7 a	5.1 q	17.2 n	14.7 o	17.8 b
	all	21.9 a	19.2 d	20.2 c	21.2 b	20.7 bc	20.6 c	22.4 a	5.7 g	18.3 e	16.0 f	
hue	1	72.8 q	77.1 hi	75.2 k−m	73.1 pq	72.7 q	72.7 q	73.5 o−q	105.0 d	78.0 h	114.3 a	81.5 c
	2	74.8 l−n	79.4 g	76.8 ij	73.9 n−p	72.9 q	77.1 hi	75.7 j−l	107.7 c	81.8 f	114.0 ab	83.5 a
	3	75.3 k−l	77.6 hi	75.8 j−l	77.6 hi	74.3 m−o	76.0 jk	74.0 n−p	101.9 e	81.3 f	113.1 b	82.6 b
	all	74.3 f	78.1 d	75.9 e	74.8 ef	73.3 g	75.3 e	74.5 f	105.0 b	80.4 c	113.8 a	
chroma	1	24.0	18.6	20.8	22.5	21.9	20.4	23.0	5.5	19.8	19.8	19.2 a
	2	22.5	20.5	21.2	22.9	21.9	21.6	23.2	7.0	18.5	16.7	19.3 a
	3	21.8	19.6	20.4	20.5	21.1	22.0	23.6	5.3	17.4	16.00	18.5 b
	all	22.8 a	19.6 e	20.8 d	22.0 b	21.6 bc	21.3 cd	23.2 a	6.0 h	18.6 f	17.5 g	

[a] *L** = lightness; *a** = green to red; *b** = yellow to blue. Hue and chroma were calculated from the *L**, *a**, and *b** values. E. Star, Eastern Star; N. Sky, Northern Sky; Fron., Frontier; H.Shot, Hot Shot; Dub., Dubloon; S. Cross, Southern Cross; Chan., Chantele; S. Gold, Solid Gold; F1 Hyb., F1 hybrid from the CSIRO melon breeding program; Del., Delicious. Data are means (n = 10). Different letters indicate a significant difference between means (P < 0.05).

Sky, Southern Cross, and the CSIRO-bred F1 hybrid) had significantly lower FRAP values in the second season (1271 μmol of Fe^{2+} kg^{-1} of FW) compared to the first (1122 μmol of Fe^{2+} kg^{-1} of FW), and melons of one cultivar (Chantele) had a greater FRAP in the second season (1370 μmol of Fe^{2+} kg^{-1} of FW) compared to the first (944 μmol of Fe^{2+} kg^{-1} of FW). There was no interaction between season and cultivar for β-carotene concentrations in fruit, with levels remaining consistent over the two seasons.

Time of sowing was a significant, but minor, source of variation for FRAP ($P < 0.001$) and β-carotene content ($P = 0.017$), accounting for approximately 3% of the variation in both cases (**Figure 2**). The average data indicated that melons harvested from vines in the second sowing were significantly different from those from both the first and third sowings, with decreased FRAP values and β-carotene contents. Fruit flesh from the second sowing had a significantly greater average hue angle and increased L^* values, indicating a move from a darker orange to a lighter orange/yellow flesh color (*22*). However, differences between sowings in FRAP values were restricted to fruit of the cultivars Northern Sky and Dubloon, whereas the FRAP values of melons of other cultivars were unaffected by sowing time. Furthermore, whereas Frontier, Dubloon, Southern Cross, and Chantele fruit all had decreased β-carotene contents in the second sowing, melons from the remaining six cultivars had consistent levels over the three sowings. Interactions between sowing date and cultivar accounted for approximately 10 and 7% of the variation in FRAP and β-carotene content, respectively.

Storage. In the first season, storage of melons at 7 °C for 4 weeks resulted in decreases in FRAP ($P < 0.001$) and β-carotene content ($P < 0.001$) (**Figure 6**). The greatest losses in FRAP and β-carotene content (30 and 36%, respectively) occurred within 2 weeks. There was little (14%) or no further significant loss in FRAP and β-carotene content, respectively, after a further 2 weeks of storage. Storage performance of melons was independent of cultivar. Cultivar $\times$ storage interactions significantly ($P < 0.001$) influenced β-carotene content and FRAP values (**Figure 6**). Spatial differences in temperature within the cold room were minimal and were therefore determined not to have contributed to variation in storage performance (data not presented).

DISCUSSION

This study has found that nutritional quality of melons can vary as a result of pre- and postharvest factors. The evidence suggests that the merit of individual melon cultivars cannot be predicted confidently by analyzing fruit from a single sowing in a single season because the metabolic pathways that lead to the production of nutritional compounds are dependent on the environment as well as genetics (see ref *33* and references cited therein). Whereas the genetic capacity of cultivars to produce nutritional compounds in their fruit contributed most, environment and associated interactions also contributed significantly to this variation (*34−36*).

We have found cultivar to be a major source of variation for β-carotene and antioxidant potential in melons. Flesh color generally reflected these nutritional indices; orange-fleshed melons possessed significantly higher levels of β-carotene and greater FRAP than either green- or white-fleshed melons (**Figure 2**). Intensely colored plant-based foods tend to be rich sources of antioxidant phytochemicals (see ref *37* and references cited therein). For example, orange-colored fruits and vegetables are high in β-carotene and red-colored fruits and vegetables are high in lycopene. Our results confirm this general linkage but also show that there is variation for β-carotene content and antioxidant potential within a group of cultivars that yield visually similar orange-fleshed melons (**Table 1** and **Figure 2**).

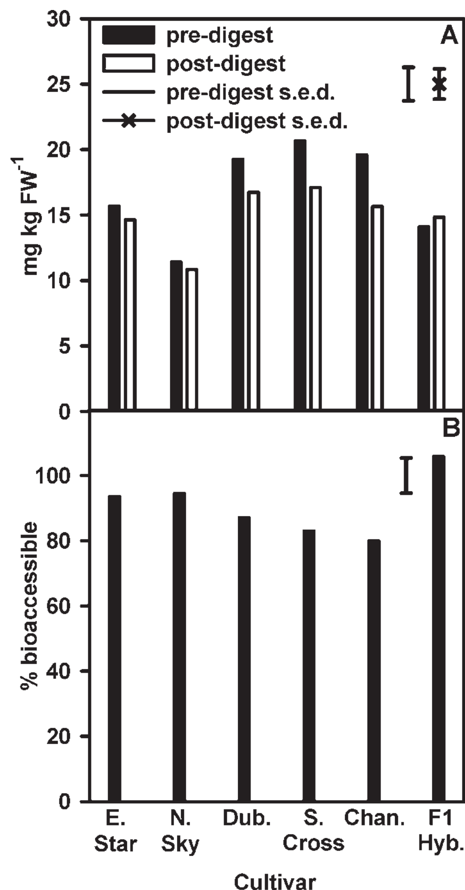

Figure 3. (**A**) β-Carotene content pre- and postdigestion and (**B**) percent bioaccessibility of β-carotene in fruit flesh sampled from melon (*C. melo*) cultivars grown in the second season (see **Table 2** for sowing date). E. Star, Eastern Star; N. Sky, Northern Sky; Dub., Dubloon; S. Cross, Southern Cross; Chan., Chantele; F1 Hyb., F1 hybrid from the CSIRO melon breeding program. Mean data ($n = 3$) are shown, and error bars are mean standard errors of difference (sed).

In addition to being a precursor of vitamin A, β-carotene also functions as a powerful antioxidant. It is most abundant in orange- and yellow-colored (carrot and sweet potato) and green leafy vegetables (spinach, broccoli), as well as fruits (apricot, sour cherry, and tomato), but in some cases only a small portion of the total carotenoid present in a given food is assimilated by the gut (*33*, *35*, *38*−*43*). The uptake of carotenoids by the gastrointestinal tract is complex and known to be influenced by numerous factors including the amount and type of carotenoid ingested, characteristics of the food matrix and other meal constituents, and the nutritional status of the consumer (*44*, *45*). The results of the in vitro assay used in the present study suggested that the β-carotene present in the melons evaluated was highly bioaccessible (**Figure 3**). As far as we are aware, there has only been one other study on the bioaccessibility of β-carotene in melons (*46*). In this case, the bioaccessibility of β-carotene in honeydew melons (assumed to have white or green flesh) was determined to be very low. However, the considerable differences in β-carotene content between white-, green-, and orange-fleshed melons render comparisons with that study to our own difficult. Additionally, in vivo studies have found that 91% of β-carotene from fruits and vegetables is available in the gut during the digestion process (*47*) and that orange-colored fruit have been found to be more effective in increasing serum concentrations of β-carotene than leafy green vegetables (*48*). These results provide some validity for the in vitro method for estimating bioaccessibility used in our study. Our results also indicated that the in vitro bioaccessibility of the β-carotene in melon, as well as the amounts present, varied according to cultivar. We also showed that these two variables were not correlated.

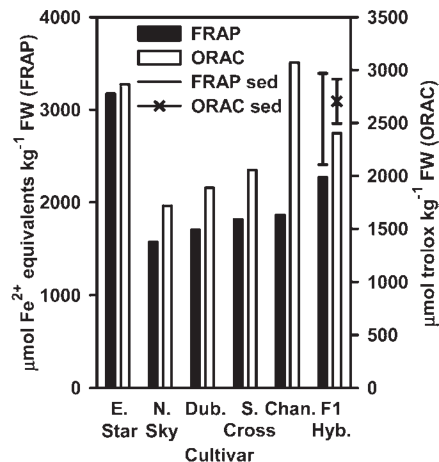

Figure 4. Comparison of the ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) in fruit flesh sampled from melon (*C. melo*) cultivars grown in the second season (see **Table 2** for sowing date). The FRAP data are from a further experiment independent from the data in **Figure 2**. E. Star, Eastern Star; N. Sky, Northern Sky; Dub., Dubloon; S. Cross, Southern Cross; Chan., Chantele; F1 Hyb., F1 hybrid from the CSIRO melon breeding program. Data are means ($n = 3$), and error bars are mean standard errors of difference (sed).

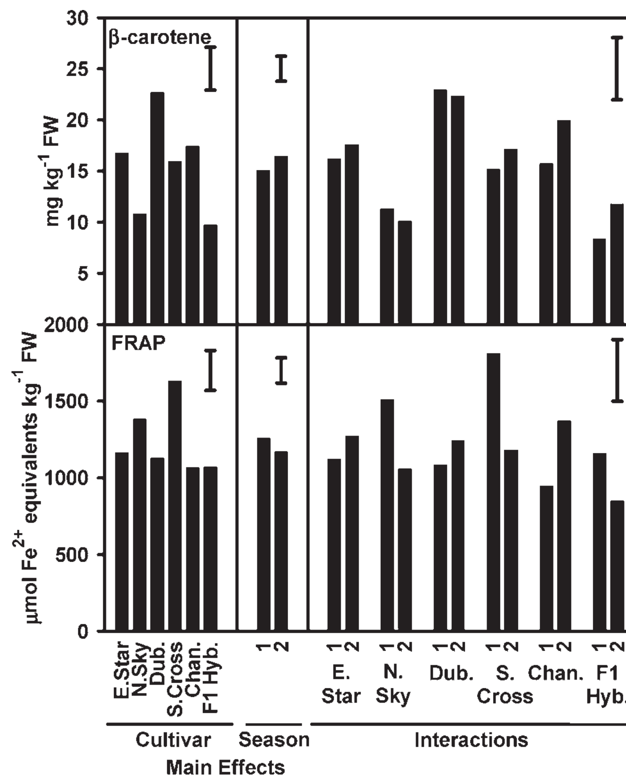

Figure 5. β-Carotene content and ferric reducing antioxidant power (FRAP) in fruit flesh sampled from melon (*C. melo*) cultivars grown over two consecutive seasons (see **Table 2** for sowing dates). E. Star, Eastern Star; N. Sky, Northern Sky; Dub., Dubloon; S. Cross, Southern Cross; Chan., Chantele; F1 Hyb., F1 hybrid from the CSIRO melon breeding program. Data are means (n = 20), and error bars are mean standard errors of difference.

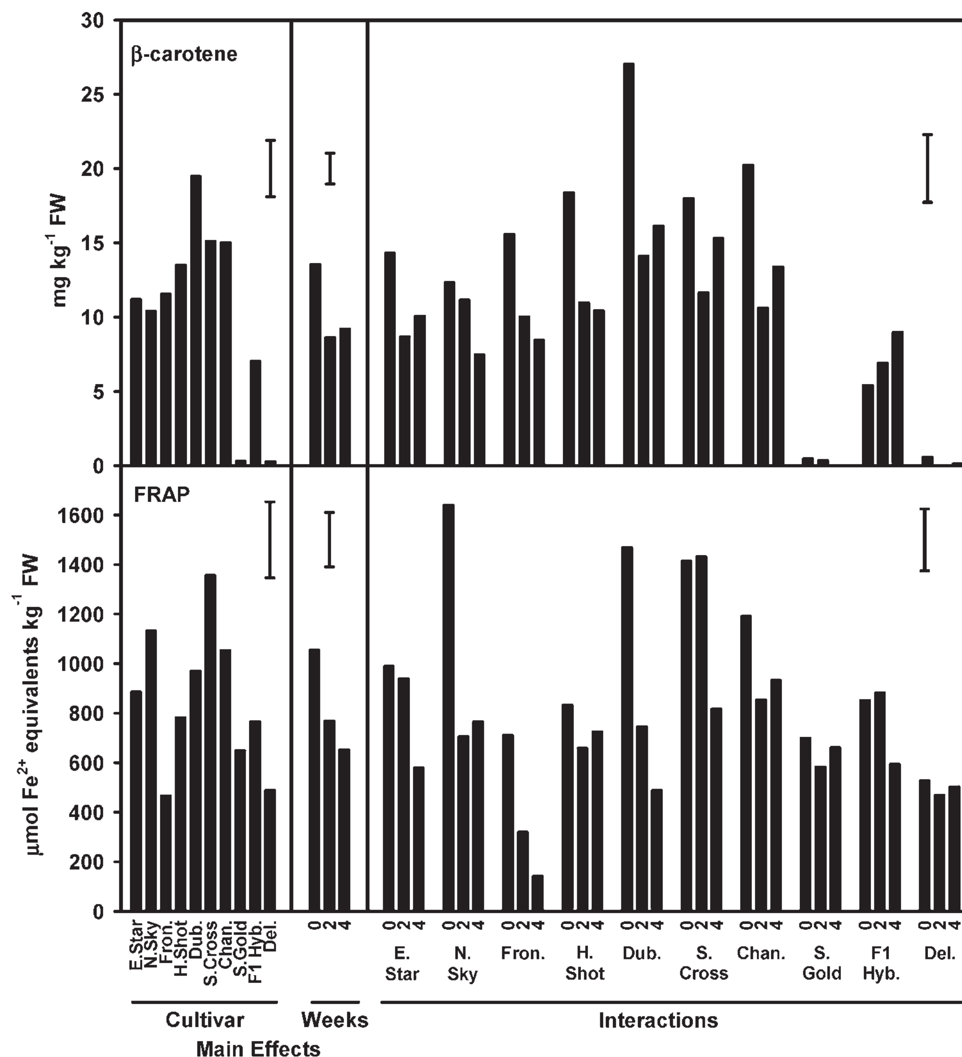

Figure 6. β-Carotene content and ferric reducing antioxidant power (FRAP) in fruit flesh sampled from melon (*C. melo*) cultivars grown in the first season (see **Table 2** for sowing dates). E. Star, Eastern Star; N. Sky, Northern Sky; Dub., Dubloon; S. Cross, Southern Cross; Chan., Chantele; F1 Hyb., F1 hybrid from the CSIRO melon breeding program. Melons were stored at 7 °C and removed for analysis at 0, 2, and 4 weeks. Data are means ($n = 5$), and error bars are mean standard errors of difference.

However, given that bioaccessibility was uniformly high among the melons of different cultivars (> 80%), breeding and selection to develop high β-carotene cultivars would be a more effective strategy for improving the nutritional quality of melons than one aimed at developing cultivars with greater β-carotene bioaccessibility.

The variation in β-carotene content of melons observed between cultivars was not reflected in the FRAP for the subgroup investigated. We found that the FRAP of green-fleshed cultivars was either equal to, or greater than, that of their orange-fleshed counterparts. Therefore, although flesh color may be indicative of carotenoid content and composition, it is clearly not a guide to total antioxidant potential of melons as measured in vitro. This may be due to higher levels of other antioxidants such as phenolic acids in green-fleshed melons. Significant amounts of total phenolic acids have been reported in colorless or green fruits and vegetables (see refs *49* and *50* and references cited therein). For example, in white carrots, carotenoids have not been detected, but there are reports that they have significant FRAP or ORAC values due to phenolic acids (*35*). Agreement between the ORAC and FRAP methods was positive for all but one cultivar, Chantelle (**Figure 4**), which was consistent with results from other studies (*51*). That the results of the two assays did not correlate may be explained by the fact that the methods used target different mechanistic aspects of antioxidant activity. The FRAP procedure measures the ability to reduce ferric ions to ferrous ions by antioxidants and equates this to antioxidant potential (*27*). The ORAC method determines the capacity of the sample to inhibit the action of peroxyl radicals and so reflects the physiologically relevant action of chain-breaking antioxidants (*51*).

This study has shown that cultivar is an important source of variation in total antioxidant potential and β-carotene content and, to a lesser extent, its bioaccessibility in melons. Additionally to this, the effects of sowing time and season were highly cultivar dependent, with cultivars interacting differently with the environment to produce changes in nutritional quality of their melons. Overall, season did not influence β-carotene content or FRAP of melons, but the FRAP values obtained for some cultivars were modulated by seasonal influences. There was a significant effect of sowing time, but it was small and highly cultivar dependent, with evidence of an interaction between cultivar and sowing time for both FRAP and β-carotene (**Figure 2**). These small changes in β-carotene content or FRAP of melons by season or sowing dates may be attributed to fluctuations in maximum and minimum

temperatures and solar radiation received in the weeks leading to harvest (**Table 2**). Such environmental factors have been shown to affect soluble solid content prior to harvest in melons (*52*) and the antioxidant potential in strawberry, *Moringa olifera*, tomato, and lettuce (*53−56*).

Whereas genetics appears to be the most important factor determining the nutritional value of melons, it does not influence nutrient retention postharvest. After 2 weeks of storage at 7 °C, reductions of 27 and 36% were observed in FRAP and β-carotene content for all cultivars (**Figure 6**). β-Carotene content decreased more dramatically than FRAP values. For example, the Southern Cross and Delicious cultivars had negligible amounts of β-carotene after 4 weeks of storage but still had significant FRAP values. This discrepancy in the results may be explained by the fact that the FRAP method is more suitable for measuring water-soluble antioxidants, such as phenolic acids (*32*) and that phenolic acids may be more stable compared to β-carotene under the storage conditions used. Therefore, it is tempting to speculate that the high FRAP values observed in some melon cultivars are related to their phenolic content. Furthermore, it has been demonstrated that total phenolic content and antioxidant activity are positively correlated (see ref *57* and references cited therein). The phenolic content in relation to FRAP/ORAC values in melon cultivars after harvest and during storage would therefore be of some interest.

On the basis of appearance and organoleptic properties (water loss, disease incidence, firmness, sugar content), the typical shelf life of a green-fleshed honeydew melon is 3−4 weeks and that for a netted melon 1−2 weeks (*58*, *59*). Therefore, our evidence suggests that loss of nutritional quality occurred before changes in appearance became apparent, potentially resulting in the ingestion of nutritionally inferior fruit.

To provide information for consumers to make informed choices and reap the full potential of horticultural products, it is essential to know the natural variation in the antioxidant contents of fruits and vegetables. In this study we have shown that melons produced from different cultivars varied significantly in antioxidant potential, and in β-carotene content and bioaccessibility, whereas sowing time and season had little effect on these variables. Therefore, selection of nutritionally superior cultivars could potentially increase the intake of health-promoting compounds and aid the prevention of human chronic diseases. Orange-fleshed melons were found to be a good source of β-carotene, the bioaccessibility of which was high. However, flesh color did not always reflect total antioxidant potential. Hence, it is erroneous to assume that the color of melon fruit is a reliable measure of its nutritional quality.

ABBREVIATIONS USED

ANOVA, analysis of variance; AUC, area under the curve; AUCblk, blank; AUCx, sample *x*; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; $FeCl_3 \cdot 6H_2O$; ferric chloride/iron chloride hexahydrate/ferric trichloride hexahydrate; FRAP, ferric reducing antioxidant power; FW, fresh weight; HPLC, high-performance liquid chromatography/chromatograph; ORAC, oxygen radical absorbance capacity; *P*, probability; REML, restricted maximum likelihood; $NaHCO_3$, sodium bicarbonate/sodium hydrogen carbonate; sed, standard error of difference; THF, tetrahydrofuran; UV−vis, ultraviolet−visible; w/v, weight per volume.

SAFETY

Tetrahydrofuran forms highly explosive peroxides if improperly stored. It is harmful by inhalation, ingestion, or skin absorption. Work within a fume hood and use protective clothing, gloves, and eyewear.

ACKNOWLEDGMENT

We thank Shona Duffield, Jacqui Fitos, and the Merbein Laboratory farm staff for the initial setup and maintenance of the trial site. We also thank Syngenta for the use of their melon cultivars.

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Received for review June 29, 2009. Revised manuscript received December 14, 2009. Accepted December 21, 2009.