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ARTICLE

Polyacetylene Diversity and Bioactivity in Orange Market and Locally Grown Colored Carrots (*Daucus carota* L.)

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Carrots contain a wide array of phytochemicals such as carotenoids, phenolics, α-tocopherol, and polyacetylenes. Carrots are most known for their pro-vitamin A carotenoids but also contain other phytochemicals with documented health benefits. The phytochemicals in colored carrots present a challenge and opportunity due to the wide diversity of potent bioactive compounds. Two commercial carrots, 1 wild carrot, and 13 colored carrot varieties were characterized phytochemically. The carrots were screened in an anti-inflammatory model of lipopolysaccharide-induced nitric oxide production. Deep Purple carrot had the highest concentration of total polyacetylenes, α-tocopherol, and total phenolics. Commercial fresh market and baby orange carrots both had high concentrations of pro-vitamin A carotenoids. Purple carrots had higher antioxidant capacity values due to their anthocyanin content. Only seven carrot varieties had inhibitory activity (IC_{25} = 257−1321 μg/mL) in macrophage cells. Among the varieties tested during the selected growing season, Deep Purple had the highest polyacetylene content and other important antioxidant phytochemicals. Further work is needed to identify other potential anti-inflammatory phytochemicals in colored carrots on the basis of this research.

KEYWORDS: Carrot (*Daucus carota* L.); colored carrots; polyacetylenes; iNOS; falcarinol; falcarindiol; falcarindiol 3-acetate

INTRODUCTION

The diversity of phytochemicals in colored carrots presents a challenge and opportunity to the health food industry promoting carrots as beneficial for human health. Colored carrots contain a diversity of carotenoids previously characterized, which include α- and β- carotene, lutein, and lycopene (*1*, *2*). Other differences in colored carrots include total phenolics, antioxidant capacity, and sensory perception (*1*−*3*).

Colored carrots also contain various amounts of polyacetylenes. Polyacetylenes are bioactive compounds that inhibit a number of enzymes such as diacylglycerol acyltransferase (DGAT) (*4*), inducible nitric oxide synthase (iNOS) (*5*, *6*), acyl-CoA:cholesterol acyltransferase (ACAT) (*7*, *8*), cholesteryl ester transfer protein (*9*), and phase II detoxification enzymes (*10*). Other experiments indicate polyacetylenes exhibit anti-inflammatory activity (*11*, *12*), are cytotoxic against cancer cell lines (*13*, *14*), and inhibit the development of induced preneoplastic colonic lesions (*15*).

Results from our laboratory recently demonstrated that polyacetylenes (falcarindiol, falcarindiol 3-acetate, and falcarinol), isolated from purple carrot, were responsible for a reduction in nitric oxide (NO) and pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) in macrophage and endothelial cells (*16*). In addition, a chromatographic fraction from purple carrot had greater bioactivity and less cytotoxicity compared to isolated polyacetylenes. In the present study, we examined the polyacetylene profile of falcarindiol, falcarindiol 3-acetate, and falcarinol in 2 commercial carrots, 1 wild carrot variety (Queen Anne's Lace), and 13 colored carrot varieties. The concentration of carrot extract, containing polyacetylenes, shown to inhibit lipopolysaccharide (LPS)-induced nitric oxide production in macrophage cells (IC_{25}) was determined in addition to the carotenoid content, α-tocopherol, total phenolics, and antioxidant activity.

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MATERIALS AND METHODS

Materials. Commercial orange table and baby carrots were purchased from a local grocer in Palmyra, WI. Queen Anne's Lace root was harvested from a local weed field. Colored carrot seeds were purchased from various commercial seed vendors (Baker Creek Heirloom Seeds, Mansfield, MO; Seed Savers Exchange, Decorah, IA; High Mowing Organic Seeds, Wolcott, VT; Johnny's Selected Seeds, Winslow, ME) and grown in raised test plots (Standard Process Inc., Palmyra, WI). Carrot seeds were sown every 2 cm in two rows (2.4 m) spaced 40 cm apart. In the fall of 2007, the carrot tops were removed followed by harvest of the mature carrots. The carrots were immediately washed, ground using a Cuisinart (East Windsor, NJ), immediately frozen, and freeze-dried (Virtis, Gardiner, NY) in 2 L flasks. After freeze-drying, the carrots were ground to a fine powder with a coffee mill, pooled, and stored at −80 °C.

Chemicals. Rutin, gallic acid, *trans*-β-apo-8′-carotenal, cerulenin, ascorbic acid, α-tocopherol, Trolox, and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Quantitation of Polyacetylenes. GC-MS analysis of carrot polyacetylenes falcarinol, falcarindiol, and falcarindiol 3-acetate were

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identified by comparison to external standards isolated by preparative HPLC and verified by GC-MS according to the methods of Czepa and Hofmann (*17*) on a HP-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness). HRGC-MS (EI, 70 eV): falcarindiol (mw 260), *m*/*z* 129 (100), 55 (89), 91 (78), 128 (75), 77 (59), 115 (56), 41 (53), 43 (45), 157 (34), 79 (34); falcarindiol 3-acetate (mw 302), *m*/*z* 43 (100), 55 (51), 128 (46), 115 (44), 129 (43), 41 (39), 91 (38), 157 (35), 77 (28), 133 (25); falcarinol (mw 244), *m*/*z* 91 (100), 55 (96), 115 (74), 117 (66), 41 (52), 129 (50), 43 (49), 131 (46) 77 (43), 159 (41). Briefly, 50 g of freeze-dried carrot was extracted in 500 mL of dichloromethane at room temperature for 2 h. After decanting, the sample was extracted with another 500 mL volume. The sample was filtered with Whatman no. 1 filter paper and rotoevaporated under vacuum to dryness at 30 °C. The sample was resuspended with sonication in a minimal amount of methanol and filtered prior to preparative HPLC.

Preparative HPLC was performed on an Agilent 1200 system equipped with an automated fraction collector. Samples were injected (900 μL) on an Agilent Eclipse XDB-C18 Prep HT (21.2 × 250 mm, 7 μm) column. The flow rate was 48 mL/min with an initial gradient of water (70%) and methanol (30%). The solvent gradient was ramped to 100% methanol at 50 min and held until 55 min before re-equilibration back to 30% methanol at 58 min. The detection wavelength was 205 nm. The collected polyacetylenes were dried under vacuum and weighed on an analytical balance to the nearest 0.1 mg.

Quantitation of polyacetylenes was performed in triplicate with the internal standard cerulenin after extraction of 1 g of freeze-dried material in 10 mL of dichloromethane followed by sonication for 15 min. After centrifugation at 4500*g*, a subsequent 10 mL dichloromethane extraction was performed. The supernatants were pooled and dried under vacuum. The dried pellet was reconstituted in 0.25 mL of dichloromethane and filtered into a GC vial. Extraction efficiency was determined to be 94.4% from the freeze-dried matrix. One microliter was injected onto the GC and compared with the respective calibration curve.

Carotenoids. Carotenoid analysis was performed according to the method developed by Horvitz et al. (*18*). Under fluorescent gold lighting 0.1 g of sample was saponified after spiking with 1 μg of the internal standard *trans*-β-apo-8′-carotenal with 150 μL of 80% methanolic KOH. The samples were saponified for 15 min at 70 °C in a water bath. After the addition of 3 mL of water, the carotenoids were extracted with 10 mL of hexane containing 0.1% butylated hydroxytoluene (BHT). The samples were centrifuged for 3 min at 1500*g* at 5 °C followed by three more extractions with 5 mL of hexane. The supernatants were combined and dried under vacuum to dryness. The samples were reconstituted in 4 mL of mobile phase A and filtered into amber HPLC vials for analysis. Forty microliters of sample was injected onto a Waters Resolve C_{18} 5 μm column, 3.9 × 300 mm, with a guard column (Milford, MA). The HPLC (Agilent Technologies) mobile phase consisted of 95:5 (v/v) acetonitrile/water with the modifiers ammonium acetate (10 mM) and triethylamine (0.1%) as solvent A and 85:10:5 (v/v/v) acetonitrile/methanol/dichloromethane, with the same modifiers, as solvent B. The gradient consisted of a 2 mL/min flow rate as follows: (1) 100% solvent A for 3 min, (2) 12 min linear gradient to 100% solvent B, (3) a 3 min hold at 100% solvent B, (4) a 1 min linear gradient back to 100% solvent A. Detection wavelengths were set to 444, 453, and 472 nm. Carotenoids were identified by comparison of their retention time and spectra with respective standards purified by HPLC immediately prior to use. Purity and concentration of the collected standards were assured by spectral analysis on a UV−vis spectrophotometer with published extinction coefficients.

α-Tocopherol. α-Tocopherol was coextracted with the polyacetylene samples as described above and eluted by the same GC method. An external calibration curve was created for quantitation based upon software integration of peak area after single ion monitoring (*m*/*z* 430).

Total Phenolics. Sample total phenolics were estimated by the Folin−Ciocalteu assay according to the method of Singleton and Rossi (*19*) and expressed as micrograms of gallic acid (GA) equivalents per gram. Briefly, 0.1 g of sample was extracted in 2.5 mL of acidified 70% acetone. A 100 μL aliquot from the supernatant was removed and diluted in 5 mL of Folin−Ciocalteu (1:10) reagent and 4 mL of a 75 g/L Na_2CO_3. After 2 h at room temperature, the absorbance was read at 765 nm with gallic acid as the standard.

Antioxidant Activity. The ferric reducing/antioxidant power (FRAP) assay was performed as previously described by Benzie and Strain (*20*). A 0.3 g sample was extracted in 10 mL of DI water with a brief vortexing and 15 min of sonication. A 1:7 dilution of the carrot extract was prepared prior to analysis. In a microplate format, 10 μL of each sample was diluted with 230 μL of the FRAP reagent that was made with 25 mL of 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-*s*-triazine (Fe^{3+}−TPTZ), and 20 mM $FeCl_3$. After intermittent shaking and incubation for 8 min at 28 °C, the plate was read at 593 nm. The final results were expressed as micromoles of ascorbic acid equivalents (equiv) per gram.

IC_{25} Determination in RAW Macrophage Cells. The concentration (μg/mL) of carrot required to inhibit 25% (IC_{25}) of the nitric oxide production was measured in LPS-induced RAW 264.7 mouse macrophage (ATCC TIB-71, Manassas, VA) cells. RAW macrophage cells were cultured in 24-well plates and treated overnight (16 h) with dried carrot extract reconstituted in the presence of 100 ng/mL LPS (Sigma, *Escherichia coli* 055:B5) in high glucose, phenol red free DMEM (Mediatech) containing 0.5% FBS, 4 mM L-glutamine, 100 units mL^{-1} of penicillin, and 100 μg mL^{-1} of streptomycin. Cells cultured in media without LPS served as a negative control. NO was determined by measuring nitrite in culture medium by the Griess reaction (*21*,*22*). Briefly, 100 μL aliquots of media were incubated with an equal volume of modified Griess reagent (Sigma-Aldrich, St. Louis, MO). After 15 min, the absorbance was measured at 540 nm using a microplate spectrophotometer (BioTek, Winooski, VT). Appropriate blanks were run by combining equal volumes of treatment media with water. Nitrite concentrations were determined on the basis of a standard curve generated with $NaNO_2$. IC_{25} values were determined in four experiments with triplicate wells.

Cell cytotoxicity was assessed with the tetrazolium salt (MTT) that is reduced by the cells to a formazan dye and measured colorimetrically (Promega, Madison, WI).

Statistical Analysis. Results are presented as mean ± standard deviation of triplicate sample analysis, unless indicated otherwise. Correlations were tried by Pearson correlation coefficient in GraphPad Prism (San Diego, CA).

Table 1. Polyacetylene Diversity in Colored Carrots

carrot variety	concentration (n = 3, μg/g, dry weight basis)			
	FaDOH	FaDOH 3-Ac	FaOH	sum
commercial baby carrots	142 ± 6	308 ± 36	236 ± 3	686 ± 43
commercial market carrots	368 ± 10	1090 ± 17	602 ± 2	2060 ± 27
Atomic Red	101 ± 7	261 ± 8	331 ± 4	693 ± 3
Snow White	82 ± 2	359 ± 15	291 ± 8	731 ± 14
Amarillo	112 ± 10	389 ± 11	257 ± 15	759 ± 35
Purple Haze	129 ± 14	339 ± 34	438 ± 68	906 ± 115
Dragon	307 ± 42	367 ± 31	369 ± 50	1043 ± 123
White Satin	182 ± 34	721 ± 40	245 ± 20	1148 ± 83
Crème de Lite	190 ± 35	678 ± 63	391 ± 41	1259 ± 106
St. Valery	228 ± 10	484 ± 21	566 ± 20	1277 ± 51
Lunar White	418 ± 24	411 ± 17	543 ± 22	1371 ± 63
Danvers	251 ± 5	605 ± 2	552 ± 9	1409 ± 15
Cosmic Purple	518 ± 7	560 ± 14	386 ± 2	1465 ± 20
Yellowstone	270 ± 26	704 ± 20	609 ± 23	1583 ± 66
Queen Anne's Lace	583 ± 26	737 ± 24	399 ± 13	1719 ± 63
Deep Purple	491 ± 10	970 ± 21	1553 ± 29	3014 ± 56

RESULTS

Of the carrot varieties studied in this experiment, Deep Purple contained the highest total amount of polyacetylenes (3014 μg/g), followed by Queen Anne's Lace (1719 μg/g) and Yellowstone (1583 μg/g). Commercial baby carrots contained the least amount of total polyacetylenes (686 μg/g). Falcarindiol was highest in Queen Anne's Lace (583 μg/g) and Cosmic Purple (518 μg/g). Falcarindiol 3-acetate was highest in Deep Purple carrot (970 μg/g) and Queen Anne's Lace (737 μg/g). Falcarinol was highest in Deep Purple (1553 μg/g) and Yellowstone carrots (609 μg/g) (**Table 1**).

The carotenoid content of the colored carrots varied as expected. Atomic Red carrots were the only ones with measurable

Table 2. Phytochemical and Antioxidant Activity Determination of Colored Carrots

	ppm ($n = 3$, μg/g, dry weight basis)						
carrot variety	lutein	α-carotene	β-carotene	lycopene	α-tocopherol	phenolics (μg of gallic acid equiv/g)	FRAP (μmol of ascorbic acid equiv/g)
commercial store carrots	43 ± 3	658 ± 181	4588 ± 1303	nd	110 ± 1	14.5 ± 0.5	23.7 ± 0.3
commercial baby carrots	76 ± 3	440 ± 111	5761 ± 1799	nd	102 ± 0	14.7 ± 0.4	23.1 ± 0.3
Amarillo	448 ± 9	nd	53 ± 32	nd	106 ± 2	2.2 ± 0.0	29.9 ± 2.5
Atomic Red	nd	nd	453 ± 176	36 ± 3	93 ± 1	9.1 ± 0.2	32.4 ± 1.0
Cosmic Purple	598 ± 123	1422 ± 407	2725 ± 321	nd	109 ± 2	3.3 ± 0.1	26.8 ± 1.3
Crème de Lite	nd	nd	nd	nd	106 ± 4	2.1 ± 0.0	9.9 ± 0.6
Danvers	316 ± 66	967 ± 302	1538 ± 476	nd	110 ± 1	8.7 ± 0.3	30.2 ± 2.1
Deep Purple	503 ± 91	35 ± 3	43 ± 2	nd	122 ± 2	35.6 ± 0.9	321.8 ± 10.1
Dragon	365 ± 65	253 ± 50	367 ± 69	nd	100 ± 1	2.6 ± 0.0	24.3 ± 1.2
Lunar White	nd	nd	nd	nd	102 ± 1	2.0 ± 0.0	13.6 ± 1.1
Purple Haze	244 ± 22	298 ± 13	3043 ± 271	nd	109 ± 5	4.1 ± 0.2	76.7 ± 5.6
Queen Anne's Lace	nd	nd	nd	nd	95 ± 1	3.2 ± 0.4	25.4 ± 0.7
Snow White	nd	nd	nd	nd	98 ± 1	2.1 ± 0.0	24.2 ± 1.2
St. Valery	70 ± 10	239 ± 78	400 ± 151	nd	105 ± 1	7.7 ± 0.8	28.2 ± 0.8
White Satin	nd	nd	nd	nd	100 ± 1	8.4 ± 0.5	23.2 ± 0.5
Yellowstone	138 ± 9	nd	nd	nd	105 ± 2	2.1 ± 0.0	15.2 ± 0.6

lycopene (38 μg/g). Both fresh market and baby carrots contained a high proportion of α- and β-carotene. Queen Anne's Lace and white carrots were devoid of carotenoids, whereas Purple Haze and Cosmic Purple carrots contained high average concentrations of lutein (99 and 299 μg/g), α-carotene (1039 and 711 μg/g), and β-carotene (1521 and 948 μg/g), respectively (**Table 2**).

α-Tocopherol levels were similar among the carrot varieties (93−110 μg/g) with the exception of Deep Purple, which was 18% higher (122 μg/g), (**Table 2**).

Total phenolics, not surprisingly, were highest in Deep Purple carrots (35.6 μg of GA/g) due to the presence of anthocyanins and other phenolic acids. Commercial market and baby carrots (14.5 and 14.7 μg of GA/g, respectively) had a higher level of total phenols compared to the average of the other colored carrot varieties (4.4 μg of GA/g). The phenolic content was lowest in white and yellow carrot varieties, with the exception of White Satin (**Table 2**).

FRAP assay results indicate that Deep Purple carrot (51.0 μmol of ascorbic acid equiv/g) and Purple Haze carrots (76.7 μmol of ascorbic acid equiv/g) had higher antioxidant potential values against iron-induced oxidation. Crème de Lite, Lunar White, and Yellowstone carrots (9.9, 13.6, and 15.2 μmol of ascorbic acid equiv/g) had the lowest antioxidant potentials, respectively (**Table 2**).

Seven of the carrot varieties exhibited inhibitory activity (IC_{25}) against NO production in LPS-stimulated macrophages. The remaining carrot varieties inhibited NO production, but only at a concentration that also affected the viability of the cells. Queen Anne's Lace had the most effective IC_{25} (257.2 μg/mL) followed by commercial market carrots, baby carrots, and Deep Purple carrot (391.9, 708.1, and 774.6 μg/mL, respectively; **Table 3**). Falcarindiol was correlated with the IC_{25} value after exclusion of baby carrots ($r^2 = 0.69$), whereas falcarinol, falcarindiol 3-acetate, and total polyacetylenes were not.

Table 3. Inhibition of Nitric Oxide Production in Macrophage Cells (IC_{25})

carrot variety	IC_{25} ($n = 4$) dry carrot equiv (μg/mL)
commercial baby carrots	708.1 ± 61.6
commercial market carrots	391.9 ± 77.3
Danvers	1321.9 ± 150.0
White Satin	1203.9 ± 47.3
Yellowstone	1110.3 ± 233.6
Deep Purple	774.6 ± 151.2
Queen Anne's Lace	257.2 ± 39.9

DISCUSSION

Carrots contain a variety of bioactive constituents including carotenoids, isocoumarins, and polyacetylenes thought to be responsible for health-promoting properties (*23*). The concentration of these phytochemicals is depedent upon many variables including the variety. The polyacetylene content in carrots studied in this experiment varied by as much as 7.1, 4.2, 6.6, and 4.4 times among falcarindiol, falcarindiol 3-acetate, falcarinol, and total polyacetylenes, respectively. The concentration of the polyacetylenes has previously been shown to vary depending on the growing conditions (*24*), cultivar (*25*), type of root tissue (*26*, *27*), and storage and processing conditions (*28*). This study demonstrates the diversity and biological activity of polyacetylenes in fresh market carrots, commercially available colored carrots, and one wild carrot. There was no control over the growing, processing, and storage conditions of the commercial carrots. Although comparison was performed on the phytochemical content, it is not a direct comparison.

The total polyacetylene contents of carrots in this study (700−3000 μg/g of dry weight) were comparable to commercial carrot determinations by others (400−1300 μg/g of dry weight) in orange market carrots assuming a water weight of 90% (*29*). The low polyacetylene content in baby carrots was expected because others have shown the compounds to be concentrated in the peel (*29*, *30*), which is removed during baby carrot processing. In the four purple carrot varieties (Purple Haze, Dragon, Cosmic Purple, and Deep Purple) falcarinol was the most abundant polyacetylene with the exception of Cosmic Purple, which has a dark orange center. In several of the white carrots (Snow White, White Satin, and Crème de Lite) and Queen Anne's Lace, the most abundant polyacetylene was falcarindiol 3-acetate.

Carotenoid makeup and concentration are what give pigmentation to the yellow, orange, and red carrot varieties. The carotenoid concentrations in this experiment are similar to those reported by others (*1*, *2*, *31*). Carotenes from carrots are an accessible form of pro-vitamin A for consumers. Purple-colored varieties such as Cosmic Purple and Purple Haze have a higher total carotene content compared to the commercially available carrots and additionally contain anthocyanin pigments. The anthocyanins in carrots have been strongly correlated with the relative antioxidant capacity (*31*). The higher antioxidant

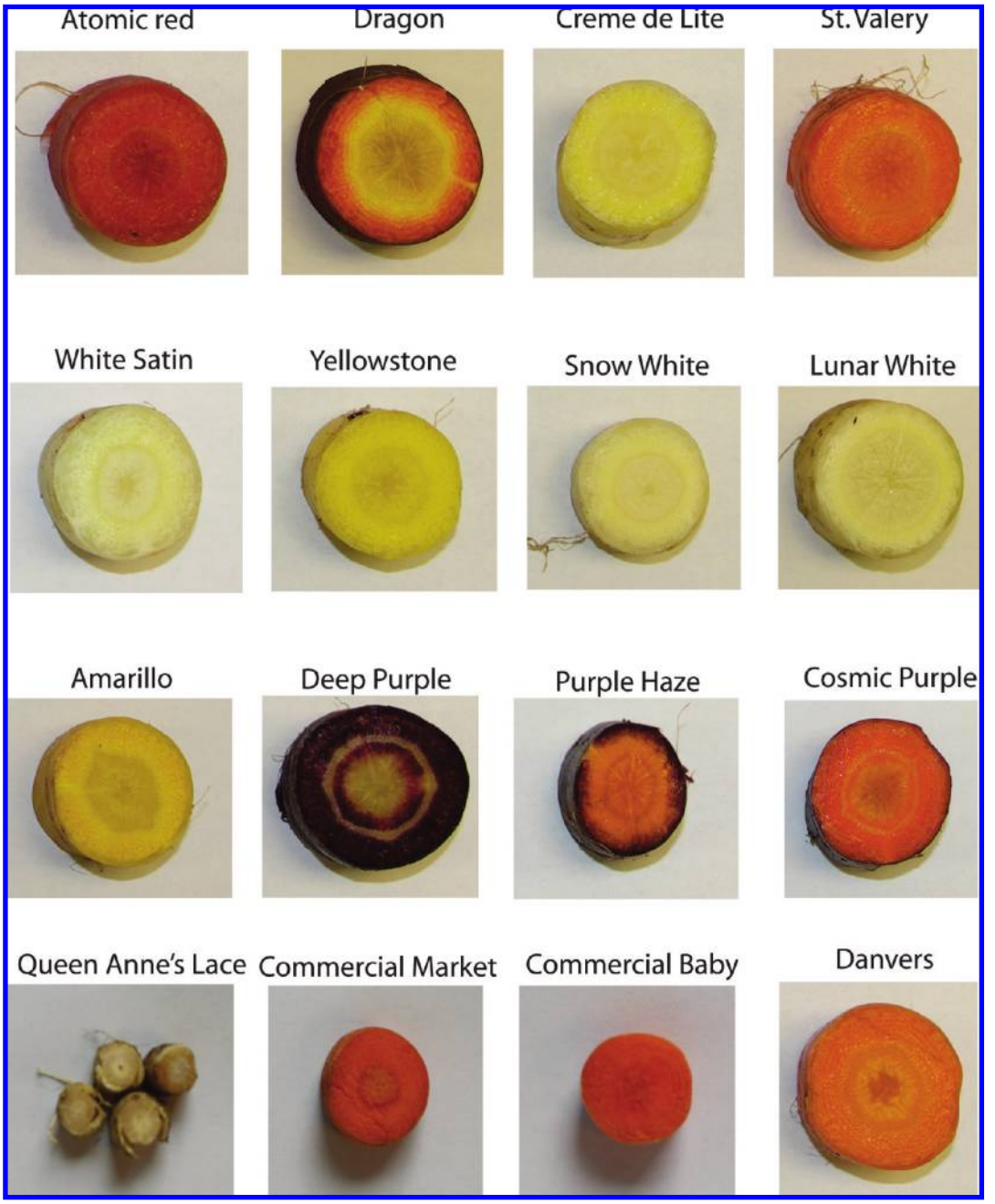

Figure 1. Colored carrot cross sections.

capacity is an additional benefit of consuming purple carrot varieties, because antioxidants scavenge free radicals that are thought to be integral to the progression of many disease states.

The diversity of antioxidants in colored carrots was apparent in this study by examination of the lipophilic antioxidant α-tocopherol and more hydrophilic phenolics, such as phenolic acids and anthocyanins. Whereas most of the carrots in this experiment were similar in α-tocopherol, Deep Purple was noticeably higher. The commercial carrots contain a high level of total phenolics compared to the colored carrot varieties with the exception of Deep Purple. Purple Haze and Cosmic Purple carrots had unexpected lower total phenolic values, but the purple anthocyanins in these varieties extend far less into the phloem from the peel in cross sections as compared to Deep Purple, which is solid to the core (**Figure 1**). The presence of anthocyanins in purple carrots was indicative of their antioxidant capacity because the more pigmented Deep Purple and Purple Haze carrots had higher FRAP values.

Our previous investigation of anti-inflammatory polyacetylenes was derived from Deep Purple carrots. In this experiment several of the colored carrots were shown to have anti-inflammatory activity. Carrots with the highest total polyacetylenes were expected to have the lowest IC_{25} values. Additionally, we previously found that falcarindiol was the most effective isolated anti-inflammatory polyacetylene in Deep Purple carrot; others have shown falcarinol to be the most cytotoxic polyacetylene (*13*). Five of the seven carrots with noted IC_{25} values had the highest content of total polyacetylene. Market baby and White Satin carrots both had lower amounts of polyacetylenes yet had bioactivity. Because the IC_{25} value was calculated only for carrots that had no effect on cell viability, it is likely that both White Satin and baby carrots reduced nitrite production and failed to inhibit cell viability due to the low falcarinol concentrations in both carrots (245 and 236 μg/g, respectively). This indicates the importance of understanding the concentrations of polyacetylenes in cell-based experiments because polyacetylenes are

hormetic phytochemicals that have been shown to stimulate cell proliferation at low concentrations and at high concentrations to inhibit proliferation (*28*). Whereas this experiment used a dichloromethane extract due to its ease of extracting a relatively pure polyacetylene fraction, our previous studies utilized a fraction from LH-20 chromatography. The LH-20 fraction used in our previous studies contained anthocyanins and was found to be less cytotoxic compared to equivalent concentrations of isolated polyacetylenes. The cytotoxicity of the polyacetylenes is noted and is known to be cell type and concentration dependent (*32*).

It is well-known that phytochemical changes can occur in carrots due to harvesting, transportation, storage, and processing (*17*, *30*, *33*), which have usually been identified through increased bitter off-taste (*34*). The polyacetylenes are one of a number of bitter components in carrots that may increase due to cold storage and processing of carrots (*23*). In this experiment the locally grown carrots were all treated the same prior to analysis. However, there was no control of the processing or development of bitter compounds in the commercial carrots that may possess anti-inflammatory activity. The development of other anti-inflammatory compounds during storage and processing would explain the bioactivity of the baby carrots despite their low polyacetylene content. 6-Methoxymellein, although not quantified, was identified by GC-MS in several carrot varieties including Queen Anne's Lace, Purple Haze, Lunar White, baby carrots, and Cosmic Purple. Besides the polyacetylenes, a number of other phytochemicals in carrot require further characterization and isolation to investigate other potential anti-inflammatory bioactivity. These include phenylpropanoids (*35*), gazarin, trans-aserone, and geraniol (*36*), galactolipids (*37*), and flavonoids and coumarins (*38*).

Among the colored carrots characterized in this study, Deep Purple contained the highest amount of polyacetylenes, α-tocopherol, and total phenolics. Deep Purple possessed one of the highest antioxidant capacity values as indicated by inhibition of ferric-induced oxidation and contains a higher percentage of anthocyanins compared to other purple carrot varieties by visual inspection. Anthocyanins also reduce nitric oxide production in macrophages (*39*), reduce chemokine, chemoattractants, and cell adhesion molecules in endothelial cells (*40*), and reduce pro-inflammatory NF-κB activation in endothelial cells (*40*,*41*). Deep Purple carrots contain a number of phytochemicals in high concentration with previously documented health benefits in comparison to other carrot varieties.

Research presented here characterizes a broad class of phytochemicals and presents bioactivity information on several colored carrot varieties with anti-inflammatory activity. The carrot, as a readily available market vegetable with previously known provitamin A carotenoids, is gaining recognition for a number of other phtyochemicals. Colored carrot varieties offer a broader range of characterized phytochemicals important in providing health benefits to the consumer.

ABBREVIATIONS USED

NO, nitric oxide; GA, gallic acid; FaDOH, falcarindiol; FaOH, falcarinol; FaDOH 3-Ac, falcarindiol 3-acetate.

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