JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Simultaneous Extraction and Quantitation of Carotenoids, Chlorophylls, and Tocopherols in *Brassica* Vegetables

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ABSTRACT: *Brassica oleracea* vegetables, such as broccoli (*B. oleracea* L. var. *italica*) and cauliflower (*B. oleracea* L. var. *botrytis*), are known to contain bioactive compounds associated with health, including three classes of photosynthetic lipid-soluble compounds: carotenoids, chlorophylls, and tocopherols. Carotenoids and chlorophylls are photosynthetic pigments. Tocopherols have vitamin E activity. Due to genetic and environmental variables, the amounts present in vegetables are not constant. To aid breeders in the development of *Brassica* cultivars with higher provitamin A and vitamin E contents and antioxidant activity, a more efficient method was developed to quantitate carotenoids, chlorophylls, and tocopherols in the edible portions of broccoli and cauliflower. The novel UPLC method separated five carotenoids, two chlorophylls, and two tocopherols in a single 30 min run, reducing the run time by half compared to previously published protocols. The objective of the study was to develop a faster, more effective extraction and quantitation methodology to screen large populations of *Brassica* germplasm, thus aiding breeders in producing superior vegetables with enhanced phytonutrient profiles.

KEYWORDS: *ultraperformance liquid chromatography (UPLC), Brassica, carotenoids, chlorophylls, tocopherols*

INTRODUCTION

Phytocompounds are primary sources of essential vitamins and nutrients necessary to support and enhance human health. *Brassica oleracea* vegetables, such as broccoli and cauliflower, contain an array of these compounds, including antioxidants, essential vitamins, and anticarcinogenic compounds, some of which are exclusive to *Brassica* species.[1,2] A number of studies have suggested that lipid-soluble antioxidants such as carotenoids, chlorophylls, and tocopherols in *Brassica* vegetables contribute to health directly through antioxidant and antimutagenic activities individually and potentially through synergistic activity with other compounds.[3−7]

Carotenoids, chlorophylls, and tocopherols are photosynthetic compounds synthesized and stored in plastidic membranes (Figure 1). Carotenoids and tocopherols are isoprenoids with a common isopentenyl pyrophosphate backbone and geranylgeranyl diphosphate precursor. Carotenoids reflect a wide range of yellow, orange, and red pigments in plants and act as accessory pigments to chlorophylls in photosynthesis. Some are also vitamin A precursors, an essential vitamin in mammalian diets. According to the World Health Organization, an estimated 250 million preschool children are vitamin A deficient (VAD) and, tragically, 250,000−500,000 of these cases progress to childhood blindness in the developing world each year.[8] Tocopherols belong to a class of tocochromanols having specific roles in plants that are not well understood, but may protect seed storage lipids from oxidation.[9] In general, plants contain higher amounts of α-tocopherol than other tocochromanols in green tissue. In *Brassica* spp., both α-tocopherol and γ-tocopherol have been identified in significant levels.[10,11]

Tocopherols have vitamin E activity in mammals, with α-tocopherol and γ-tocopherol exhibiting the greatest effects.[12] These activities involve protecting membrane lipids from oxidative damage by quenching reactive oxygen and nitric oxide species,

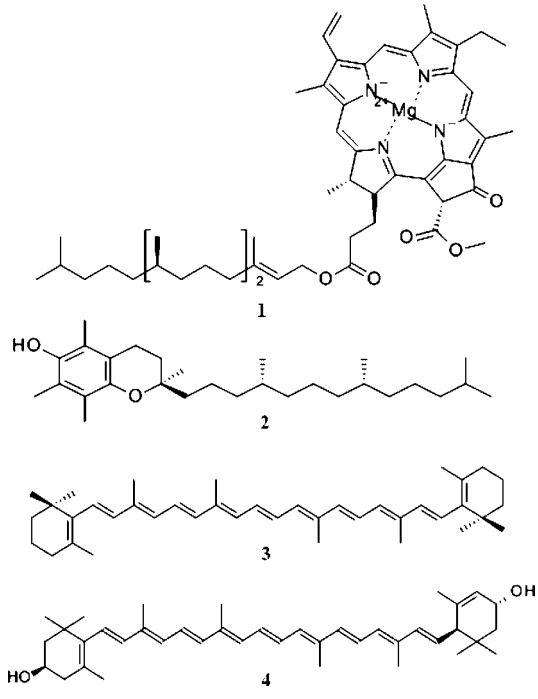

Figure 1. Compound structures for (**1**) chlorophyll *a*, (**2**) α-tocopherol, (**3**) β-carotene, and (**4**) lutein.

acting as anti-inflammatory agents, and protecting against degenerative diseases.[13−18] Chlorophylls are also photosynthetic plastidic compounds containing a porphyrin ring with an attached

Received: March 6, 2012
Revised: June 25, 2012
Accepted: June 27, 2012
Published: June 27, 2012

magnesium and a phytol tail derived from the isoprenoid pathway (Figure 1).[19] The two major chlorophylls (*a* and *b*) are green pigment photoreceptors present in all photosynthetic organisms. Recent studies suggest that they contribute to antioxidant, antimutagenic, and antiviral activities.[20−22]

Due to the lipophilic nature of these compounds and the high rate of oxidation, the extraction and quantitation of carotenoids, chlorophylls, and tocopherols has required labor-intensive, multistep protocols. However, this can increase their exposure to oxygen and warm temperature, potentially introducing oxidative damage to carotenoids, chlorophylls, and tocopherols. Published extraction procedures for *Brassica* include saponification of extracts and the use of multiple solvents in lengthy separation methods.[10,11,23−25] Saponification of extracts with a strong hydroxide in the presence of high temperatures has been employed to accurately identify esterified xanthophylls; however, the same process will likely degrade unesterified carotenoids such as *β*-carotene.[26,27] In *Brassica* vegetables the xanthophylls are predominately free nonesterified structures; therefore, saponification is not essential and may lead to inaccurate quantitation. In light of this, previous studies utilizing saponification in *Brassica* vegetables have likely underestimated the phytochemical concentrations present in edible portions.

Additionally, commonly used high-performance liquid chromatography (HPLC) methods in *Brassica* spp. often consist of run times lasting from 40 to 60 min with flow rates as high as 2 mL/min and can result in poor resolution of some carotenoids.[10,11,23−25] These lengthy methods make them inefficient for the processing of large numbers of samples.

Previous reports quantitating lipid-soluble compounds in *B. oleracea* vegetables have been inconsistent.[10,11,24,28] This is likely attributable in part to differences in genotypes, environments, and postharvest handling of the material in the various studies but also due to differences in extractions and analytical methodology. As public and private breeding programs respond to the consumer demand for healthier fruits and vegetables, the need for high-throughput, consistent, and multiplexed analyses to analyze large numbers of samples has developed. This paper offers a novel method for simultaneously extracting and analyzing carotenoids, chlorophylls, and tocopherols from *Brassica* vegetables that is faster and more accurate than previously described methods.

MATERIALS AND METHODS

Sample Preparation and Extraction. Seven *B. oleracea* genotypes (four broccoli types and three cauliflower types) were selected and analyzed in this study. Three broccoli varieties (*B. oleracea* L. var. *italica*), 'VI-158', 'BNC' ('Brocolette Neri E. Cespuglio'), and 'Pirate', were grown at the North Carolina Department of Agriculture, Piedmont Research Station, in Salisbury, NC, USA, during the fall and spring of 2009 and 2010, respectively. A purple-headed broccoli, 'Violet Queen' (Takii; Salinas, CA, USA) was grown and harvested in Charleston, SC, USA, as well as three cauliflowers (*B. oleracea* L. var. *botrytis*), 'Snowball', a white cauliflower (Ferry-Morse; Fulton, KY, USA); 'Cheddar', an orange cauliflower (Seminis; St. Louis, MO, USA), and 'Graffiti', a purple cauliflower (Seminis). All material from Charleston was provided courtesy of Dr. Mark Farnham (USDA-ARS).

B. oleracea floret and curd tissue was flash frozen in liquid nitrogen, stored at −80 °C until lyophilization at −30 °C, ground to a very fine powder using a coffee bean grinder, and stored at −20 °C in the dark until further use. Under yellow lights, 0.2 g of freeze-dried finely ground tissue was extracted with 5 mL of pure ethanol and shaken for 20 min on ice at 180 rpm. Three extractions with ethanol produced a color-free pellet. After each extraction, the extracts were centrifuged at 2900g_n for 15 min at 10 °C to collect the supernatant. The supernatants were pooled, completely dried under nitrogen gas, and resuspended in 1.5 mL of ethanol. The ethanolic extract was filtered through a 0.2 *μ*m polytetrafluoroethylene filter (Nalgene, Rochester, NY, USA) into an amber HPLC vial and stored under nitrogen gas at −80 °C until analysis. Triplicate extractions were performed for each sample.

Calibration verification, internal standards, and method verification standards were prepared by spiking the 'BNC' lyophilized floret tissue powder. The calibration verification standard validates the accuracy of the sample preparation and is used for recovery analysis. Three samples were prepared by adding 10 *μ*g of apo-carotenal internal standard to 0.2 g of 'BNC' powdered lyophilized broccoli floret tissue. The tissue was extracted and filtered into an amber vial.

Triplicate 'BNC' samples containing 0.2 g of 'BNC' tissue and an additional 85 *μ*g of *β*-carotene standard were extracted and used as method verification samples. Due to the prohibitive cost of most pure standards, *β*-carotene was chosen as a representative standard to spike sample tissue. Comparing detection signals and choosing a signal comparable to the endogenous metabolites determined the amount of *β*-carotene added. These samples were used for overall accuracy of the chromatographic method by validating retention time (t_R) stability.

External Standard Preparation. Chlorophyll *a*, chlorophyll *b*, *α*-tocopherol, *γ*-tocopherol, *β*-carotene, and apo-carotenal standards were purchased from Sigma, whereas carotenoid standards (lutein, lutein epoxide, violaxanthin, and neoxanthin) were purchased from CaroteNature GmbH (Lupsingen, Switzerland). Chlorophylls and tocopherols were easily dissolved in ethanol. Carotenes and xanthophylls were initially dissolved in ethanol and tetrahydrofuran, respectively, and then diluted in hexane. Six serial dilutions were made for each, and standard dilution concentrations were determined spectrophotometrically using their respective molar extinction coefficients.[27] Immediately afterward, 5 *μ*L of each standard was injected to detect corresponding retention time and spectra for each standard as criteria for proper peak identification. Carotenoid standard concentrations ranged from 7.13 to 228.0 *μ*g/mL, chlorophyll standard concentrations ranged from 31.5 to 1000 *μ*g/mL, and tocopherol standard concentrations ranged from 25.0 to 411.0 *μ*g/mL. Calibration curves and linear regression equations were generated for each external standard.

Chromatographic Conditions. For the characterization of carotenoids, chlorophylls, and tocopherols, a Waters ACQUITY ultra-performance liquid chromatography (UPLC) unit with a photodiode array (PDA) detector was used. The column used was a 2.1 mm × 100 mm i.d., 1.8 *μ*m, Waters ACQUITY HSS T3 (Waters, Milford, MA, USA) set at 35 °C. The solvent system consisted of 0.05% ammonium acetate (A) and 74:19:7 (v/v/v) acetonitrile/methanol/chloroform (B). The profile included two linear phases (0−18 min at 75% B; 18−19 min from 75 to 100% B; 19−30 min from 100 to 98% B). The flow rate was set at 0.4 mL/min, and the injection volume was 5 *μ*L. Spectra were observed for the wavelengths between 240 and 670 nm. Carotenoids were detected at 450 nm, chlorophylls at 650 nm, and tocopherols at 292 nm and identified according to t_R and spectra comparison.

Data Analysis. A calibration curve and linear regression equation were determined for each external carotenoid, chlorophyll, and tocopherol standard using six dilutions per standard. The injection volume was 5 *μ*L. Injection percent recovery was calculated using the calibration verification standard consisting of 'BNC' samples spiked with known concentration of apo-carotenal and *β*-carotene internal standards. Recovery was calculated by $R\ (\%) = [(C_s - C_p)/C_a] \times 100$, where R (%) is percent recovery, C_s is total carotenoid content in the spiked sample, C_p is endogenous carotenoid content in the sample, and C_a is the amount of carotenoid standard added to the sample.

The method verification standard (MVS) of 'BNC' spiked with a known amount of *β*-carotene was used to calculate percent *β*-carotene recovery after extraction and chromatography. The initial spiked *β*-carotene amount was corrected and subtracted from the actual observed concentration in MVS, divided by the concentration of the *β*-carotene spike, and multiplied by 100 to yield percent recovery of MVS. Every sample was extracted in triplicate, and each was injected three times. All sample concentrations and standard deviations are reported in micrograms per gram of dry weight (DW).

For each carotenoid, chlorophyll, and tocopherol standard, λ_{max} values were determined spectrophotometrically (Table 1). In addition, the % III/II was calculated for carotenoids to give an idea of the spectroscopic fine structure for each in their corresponding solvents. The sensitivity of the chromatographic method was measured by calculating the limits of detection (LOD) and quantitation (LOQ) for each metabolite measured in the samples (Table 1).

Table 1. Limits of Detection and Quantitation for Standard Compounds

standard compound	solvent	λ_{max}	% III/II	LOD[c]	LOQ[c]
carotenoids					
neoxanthin	hexane[a]	445	61	15.0	30.2
violaxanthin	hexane[a]	430	68	7.38	21.3
lutein epoxide	hexane[a]	446	80	12.3	36.4
lutein	hexane[a]	454	58	18.7	54.0
β-carotene	hexane[b]	452	37	7.36	20.0
apo-carotenal	hexane[b]	455	8	4.1	9.6
tocopherols					
γ-tocopherol	ethanol	297	NA[d]	3.35	10.2
α-tocopherol	ethanol	292	NA	33.3	90.1
chlorophylls					
chlorophyll *a*	ethanol	664	NA	30.7	93.1
chlorophyll *b*	ethanol	649	NA	18.5	46.1

[a]Standard initially dissolved in ethanol, followed by dilutions in hexane. [b]Standard initially dissolved in tetrahydrofuran, followed by dilutions in hexane. [c]Amounts reported in μg/mL. [d]NA, not applicable to the compound.

RESULTS AND DISCUSSION

Extractions and Chromatographic Conditions. An ethanolic extraction procedure was used to simultaneously extract carotenoids, chlorophylls, and tocopherols from freeze-dried tissues. Compared to previous extraction methods, this extraction protocol was faster and did not include a saponification step. Extracts were not exposed to high temperatures, strong basic solutions, or oxygen for long periods, thereby decreasing the chances of degradation due to oxidation. As shown in Figures 2 and 3, the described chromatographic method resulted in the most efficient separation of three classes of lipophilic compounds, carotenoids, chlorophylls, and tocopherols, in 30 min. Previous *Brassica* studies had shown the separation and quantitation of only one or two of the three classes of compounds studied in the present work and used much higher flow rates.[10,11,23,25,28] Burns et al.[24] had shown the separation of all three classes of compounds in broccoli; however, the run time was lengthy at 54 min, and the flow rate was 1 mL/min. External standards were analyzed as shown in Figures 2C,D and 3C, showing the effective separation and retention times of six carotenoids, two chlorophylls, and two tocopherols most commonly present in *Brassica* vegetables.

The chromatograms at 450 nm from the broccoli extracts contained more peaks than those of cauliflower (Figure 2A,B). By comparison of retention times and spectra of the peaks observed at 450 nm with the external standards, all peaks were characterized as free nonesterified carotenoids. The lack of extra peaks indicates that there were no esterified carotenoids present, because these would elute at retention times different from that of the parent compound. The lack of unidentified peaks also demonstrates that there was no carotenoid, chlorophyll, or tocopherol degradation during the extraction procedure with ethanol, as this would have resulted in additional unidentified peaks.

Method Validation. Apo-carotenal was used as an internal calibration verification standard with a percent recovery calculated by using the apo-carotenal standard curve. Figure 2C illustrates apo-carotenal eluting at 21.6 min. After triplicate spiked samples were injected three times, recovery of the initial apo-carotenal amount added to the spiked broccoli sample prior to extraction and chromatographic procedures resulted in a 92 ± 5% recovery. Retention time stability was validated by adding a known amount of β-carotene standard to a known amount of broccoli tissue, triplicate samples and triplicate injections. After extraction and chromatography, all β-carotene eluted at the same retention time and 95 ± 3% of the added β-carotene standard was recovered.

Carotenoid Quantitative Analysis. The described ethanol extraction and 30 min chromatographic methods proved to efficiently extract and separate nine prominent metabolites in *Brassica* vegetables; amounts are in Table 2. As expected, carotenoids were not detected in white cauliflower, whereas neoxanthin was found in purple cauliflower. The detection of neoxanthin is an indication that purple cauliflower does produce carotenoids, but only accumulates neoxanthin, which occurs downstream in the carotenoid biosynthetic pathway.

'Cheddar' cauliflower accumulated three carotenoids, neoxanthin, violaxanthin, and β-carotene (Figure 2B), which made up over half of the total carotenoids present (Table 2). Previous studies reported a different orange cauliflower variety with β-carotene amounts ranging from 5 to 8 μg/g fresh weight (FW) (approximately 33−53 μg/g DW) and negligible amounts of neoxanthin, violaxanthin, and lutein.[29] In the current study, β-carotene (86.8 ± 5.0 μg/g DW), neoxanthin (40.2 ± 0.5 μg/g DW), and violaxanthin (19.9 ± 0.2 μg/g DW) were quantified (Table 2). The three reported carotenoids were the only detected peaks found in 'Cheddar' cauliflower (Figure 2B), suggesting that only carotenoids branching from the β-carotene pathway accumulated, whereas no lutein or lutein epoxide was found. White cauliflower curd, unlike broccoli florets, is not photosynthetic tissue and does not contain chloroplast or chromoplasts, the structures needed to sequester carotenoids. In orange cauliflower curd tissue, the presence of carotenoids is the result of the *Or* gene mutation responsible for the formation of chromoplasts.[30]

In photosynthetic broccoli floret tissue, carotenoids are synthesized and stored in chloroplasts, where they act as accessory pigments to chlorophylls in photosynthesis. Due to the high concentration of chlorophyll found in broccoli florets, the appearance of all broccoli tissue was green, even though multiple yellow and orange pigmented carotenoids were present in all four broccoli varieties (Table 2). Overall, the variety with the highest amount of total carotenoids was 'BNC' with 367.6 ± 15.7 μg/g DW. The highest amount of the provitamin A carotenoid, β-carotene, was 73.5 ± 3.6 μg/g DW also in 'BNC', whereas the lowest amount was found in the purple broccoli parent with 44.5 ± 4.3 μg/g DW. A previous study by Burns et al.[24] quantified much lower amounts of β-carotene in broccoli; this could be due to possible degradation during their extraction procedure considering it was lengthy and involved a saponification protocol. In all broccoli varieties, the predominant carotenoid was lutein, which varied 2-fold among the broccoli varieties in this study; 'BNC' accumulated the highest with 120.5 ± 7.7 μg/g DW. Contrary to our findings and those of others,[28] Ibrahim and Juvik[10] reported β-carotene amounts consistently higher than

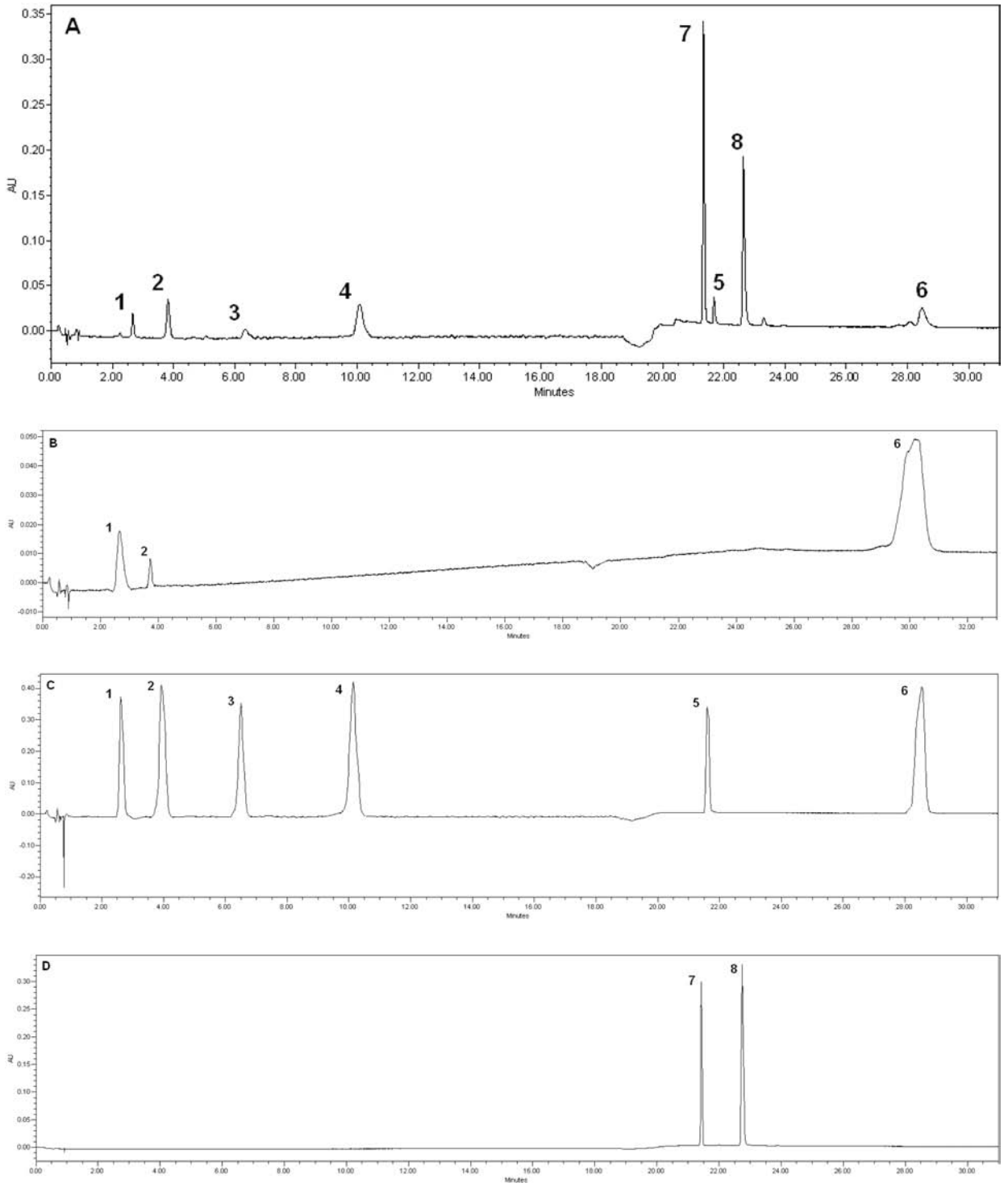

Figure 2. Carotenoid and chlorophyll peaks identified and numbered 1−8: (A) chromatogram of a mixture of carotenoids and chlorophylls 'BNC' broccoli extract spiked with apo-carotenal internal standard at 450 nm; (B) chromatogram of 'Cheddar' cauliflower extract at 450 nm (three carotenoids identified); (C) chromatogram of six carotenoid standards at 450 nm (1, neoxanthin (2.7 min); 2, violaxanthin (3.8 min); 3, lutein epoxide (6.5 min); 4, lutein (10.1 min); 5, apo-carotenal (21.6 min); and 6, β-carotene (28.5 min)); (D) chromatogram of two chlorophyll standards at 650 nm (7, chlorophyll *a* (22.7 min); and 8, chlorophyll *b* (21.5 min)).

lutein amounts. This could have been due to carotenoid oxidation during the extraction process or to poor chromatographic quantitation. In contrast to Farnham and Kopsell,[28] in the current study lutein epoxide was identified, whereas antheraxanthin was not.[28] However, these compounds are both xanthophyll epoxides and have similar chemical properties, making it difficult to separate and identify accurately without mass spectrometry.[31] As these compounds occur in relatively minor amounts in *Brassica* vegetables and have been shown to have little bioavailability, the discrepancy is unlikely to have an impact on human health.[32]

Tocopherol Quantitative Analysis. At 292 nm, α- and γ-tocopherols were detected (Figure 3), and all *Brassica* vegetables accumulate both tocopherols as shown in Figure 3AB and

dx.doi.org/10.1021/jf302475d | J. Agric. Food Chem. 2012, 60, 7238−7244

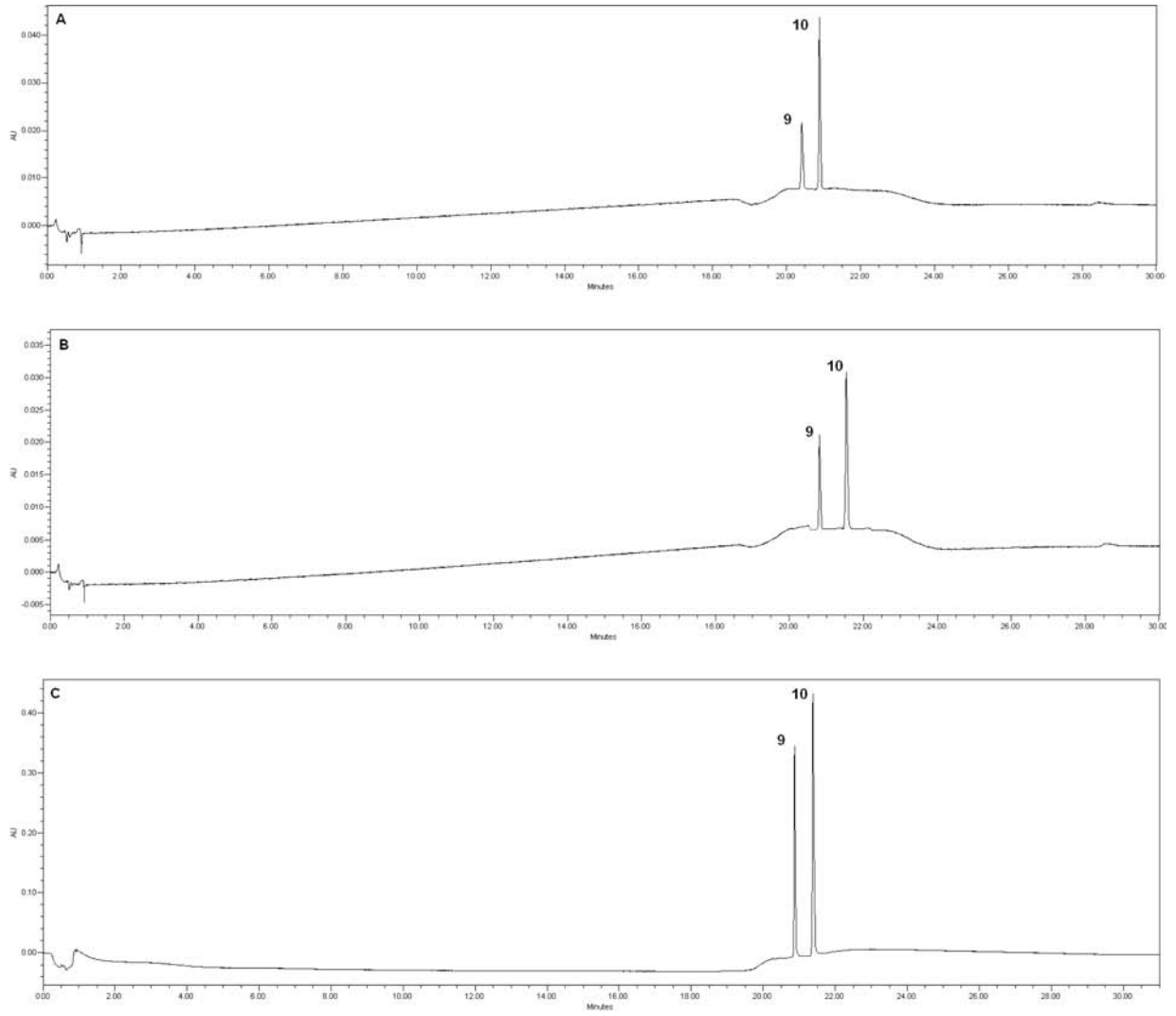

Figure 3. Tocopherol peaks identified and numbered 9 and 10: (A) chromatogram of 'BNC' broccoli extract at 292 nm; (B) chromatogram of purple cauliflower extract at 292 nm; (C) chromatogram of two tocopherol standards at 292 nm (9, γ-tocopherol (20.8 min); and 10, α-tocopherol (21.5 min)).

Table 2. Carotenoid, Chlorophyll, and Tocopherol Amounts for *B. oleracea* Vegetables[a]

	cauliflower			broccoli			
compound	white	'Cheddar'	purple	'VI-158'	'BNC'	purple parent	'Pirate'
γ-tocopherol	61.5 ± 6.3	65.8 ± 0.9	84.2 ± 10.0	89.1 ± 12	76.4 ± 3.0	93.6 ± 11.0	50.9 ± 1.7
α-tocopherol	151.4 ± 1	151.4 ± 0.5	152.5 ± 0.3	209.6 ± 16.9	181.8 ± 2.0	177.6 ± 6.4	264.5 ± 17.1
total tocopherols	212.9 ± 7.2	217.2 ± 1.5	236.6 ± 10.3	298.7 ± 28.9	258.2 ± 5.0	271.3 ± 17.4	315.4 ± 18.8
neoxanthin	ND[b]	40.2 ± 0.5	36.5 ± 0.4	50.4 ± 4.1	58.7 ± 1.5	43.4 ± 2.6	50.4 ± 3.8
violaxanthin	ND	19.9 ± 0.2	ND	52.3 ± 9.0	63.9 ± 2.2	28.2 ± 3.5	47.7 ± 5.6
lutein epoxide	ND	ND	ND	56.6 ± 7.5	50.9 ± 0.7	42.6 ± 3.2	46.8 ± 4.1
lutein	ND	ND	ND	90.0 ± 17.0	120.5 ± 7.7	64.1 ± 10.5	101.8 ± 12.3
β-carotene	ND	86.8 ± 5.0	ND	59.2 ± 9.5	73.5 ± 3.6	44.5 ± 4.3	60.1 ± 4.5
total carotenoids	ND	146.8 ± 5.6	36.5 ± 0.4	308.5 ± 47.1	367.6 ± 15.7	222.8 ± 24.1	306.8 ± 30.3
chlorophyll *a*	ND	ND	ND	521.4 ± 23.6	936.3 ± 31.3	266.9 ± 16.9	457.2 ± 12.3
chlorophyll *b*	ND	ND	ND	115.3 ± 2.4	209.0 ± 10.7	55.1 ± 2.3	114.8 ± 3.9
total chlorophylls	ND	ND	ND	636.8 ± 26.0	1145.3 ± 42	322.0 ± 19.2	572.0 ± 16.2

[a]Amounts are reported in μg/g DW ± standard deviation, where n = 3. [b]ND, not detectable.

Table 2. Traditionally, it was suggested that vitamin E activity is highest with α-tocopherol, but recent studies indicate that both α- and γ-tocopherol exhibit vitamin E activity.[12−18] Table 2 shows that α-tocopherol accumulated at least 2-fold compared to γ-tocopherol in all *Brassica* vegetable samples. The broccoli cultivar 'Pirate' contained the highest amount of α-tocopherol,

264.5 ± 17.1 μg/g DW. Ibrahim and Juvik[10] reported a considerably lower amount, approximately 60 μg/g DW in 'Pirate'.[10] For the broccoli 'VI-158', Kurilich et al.[11] also reported an α-tocopherol amount lower in comparison to the amount found in the current study. Both of these studies[10,11] used a saponification process that involved incubating the extract in 80% KOH at 70 °C for 30 min, whereas the current study did not use a saponification procedure.[10,11] By comparison of cauliflower and broccoli samples, all three cauliflower varieties had similar amounts of total tocopherols, which was less than the broccoli varieties. Total tocopherol amounts varied from 212.9 ± 7.2 to 315.4 ± 18.8 μg/g DW in white cauliflower and 'Pirate', respectively, with 'VI-158' also accumulating high total tocopherol amounts of 298.7 ± 28.9 μg/g DW. Considering that α-tocopherol has the highest vitamin E activity, a vegetable with higher tocopherol amount in the form of α-tocopherol is preferred.

Chlorophyll Quantitative Analysis. Chlorophyll *a* and *b* peaks were detected at 650 nm (Figure 2D). Even though their maximun absorption is near 650 nm (Table 1), Figure 2A shows two peaks for chlorophylls *a* and *b* at 450 nm because chlorophylls are known to absorb some light at 450 nm. White, purple, and 'Cheddar' cauliflower displayed no detectable chlorophyll peaks, whereas both peaks were present in all four broccoli varieties. As 'Cheddar' cauliflower has no green chloroplasts, its orange appearance is due as much to an increase in overall carotenoid biosynthesis as it is to carotenoid sequestration in chromoplasts, without the masking effects of chlorophyll in chloroplasts.

It has been reported that the ratio between chlorophylls *a* and *b* is a minimum of three to one in green leaf tissue and broccoli florets.[19,20,33] In our study, the four broccoli varieties screened contained chlorophyll *a* levels 4 times that of chlorophyll *b*. In broccoli, total chlorophyll amounts ranged from 322.0 ± 19.2 (purple broccoli 'Violet Queen') to 1145.3 ± 42 μg/g DW ('BNC'). Although the tissue material came from different *B. oleracea* cultivars, Farnham and Kopsell[28] also reported total chlorophyll amounts ranging from 290.0 to 922.6 μg/g DW; however, unlike the present study, chlorophyll *a* concentrations in the previous study were <2 times higher than chlorophyll *b* concentrations.

In conclusion, the extraction and chromatographic methods presented in this study proved to be a fast, efficient system for the simultaneous analysis of carotenoids, chlorophylls, and tocopherols in *Brassica* vegetables. Genetic differences among the cultivars produced dramatic and unexpected results in metabolite profiles. In 'Cheddar' cauliflower, the color was due to levels of the orange pigment β-carotene and not to an accumulation of yellow pigments such as lutein. Variation of carotenoid amounts was also seen from one broccoli variety to another, suggesting that significant progress can be made in breeding broccoli and cauliflower varieties with enhanced or modified profiles. This method will facilitate screening larger populations of *Brassica* vegetables in a more efficient manner while ensuring that resolution and quantitative accuracy are not compromised.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the North Carolina Department of Agricultural, Piedmont Research Station in Salisbury, North Carolina, and Mark Farnham for providing some of the broccoli and cauliflower tissues used in this study.

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