Food Chemistry Advances 2 (2023) 100178

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

Spectral properties and stability of selected carotenoid and chlorophyll compounds in different solvent systems ☆

Eyosias L. Ashenafi [a], Marianne C. Nyman [a,*], Jacob T. Shelley [b], Neil S. Mattson [c]

[a] Department of Civil and Environmental Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[b] Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[c] School of Integrative Plant Science, Cornell University, Ithaca, NY 14583, USA

ARTICLE INFO

Keywords:
Plant pigments
Stability
UV-VIS
Chlorophylls
Carotenoids

ABSTRACT

A quick, accurate, and low-cost analytical method for the assessment of bioactive leaf pigments, such as carotenoids and chlorophylls, can be beneficial for plant growers and consumers. The objective of this study was to learn about the thermal stability of major plant pigments and investigate the potential for rapid extraction and quantification of individual compounds in leafy greens. In this study, the spectral properties and chemical stability of violaxanthin, neoxanthin, lutein, zeaxanthin, β-carotene, and chlorophyll *a* and *b* pigments were examined in different solvent systems. Within the same solvent system, bathochromic shifts were observed in the analysis of carotenoids that contain a higher number of conjugated double bonds. Depending on the solvent system, pigments exhibited different degrees of stability. The results from this study can be used to identify an ideal solvent system for the storage of pigment standards and for the analysis of these pigments in leaf samples using UV-VIS spectroscopy.

1. Introduction

Carotenoids are a family of naturally occurring C_{40} isoprenoid compounds that are synthesized by microorganisms, fungi, and plants (DellaPenna, 1999; Hopkins & Hüner, 2009; McDonald, 2003). Over 750 carotenoids have been isolated and identified in plants, algae, and bacteria (Lohr & Wilhelm, 1999; Rodriguez-Amaya, 2016). In nature, most carotenoids exist in the all-*E* geometric conformation. Transformation or isomerization of all-*E* carotenoids results in *Z*-isomeric forms during heating and exposure to light (Rodriguez-Amaya & Kimura, 2004). Carotenoids with *Z*-geometry have strong characteristic light absorption in the 330 - 350 nm wavelength region (Schieber & Carle, 2005; Tsao & Deng, 2004).

Xanthophyll carotenoids, including lutein and zeaxanthin, are soluble in polar solvents, while carotenes (β-carotene, lycopene, etc.) are soluble in non-polar solvents. Acetone and hexane are widely used solvents for plant pigment analysis for polar and non-polar carotenoids, respectively, due to the high extraction efficiency and stability of the pigments in these solvents (Amorim-Carrilho et al., 2014; Pocock et al., 2004; Saini & Keum, 2018). Craft and Soares (1992) reported the solubility of lutein as 800 mg L^{-1} in acetone and 20 mg L^{-1} in hexane, while the solubility of β-carotene was 200 mg L^{-1} in acetone and 600 mg L^{-1} in hexane. These researchers found the highest solubility of lutein and β-carotene in tetrahydrofuran, chloroform, and dichloromethane solvents. However, these solvents are toxic to humans and to the natural environment (Joshi & Adhikari, 2019). In addition, these solvents can lead to carotenoid degradation or loss due to peroxide formation (Craft & Soares, 1992).

In some instances, pigment precipitation or crystallization in different solvents due to low solubility has been previously reported. Examples include lycopene in methanol, zeaxanthin in hexane and cyclohexane, and β-carotene in methanol, acetonitrile, and 70:30 and 80:20 (v/v) mixtures of acetone and DI water (Craft & Soares, 1992; Barba et al., 2006; Paradisoet al., 2020; Popova, 2017; Zang et al., 1997).

Overall, there is limited scientific literature on the stability of bioactive pigment molecules, such as carotenoids, in pure or natural matrices

Abbreviations: acetone:deionized water, (ACE:DI); methanol:DI water, (MeOH:DI); acetonitrile:DI water, (ACN:DI); acetone:methanol:DI water, (ACE:MeOH:DI).

☆ Chemical compounds studied in this article: Lutein (PubChem CID: 5281243); β-Carotene (PubChem CID: 5280489); Neoxanthin (PubChem CID: 5282217); Violaxanthin (PubChem CID: 448438); Zeaxanthin (PubChem CID: 5280899); Chlorophyll a (PubChem CID: 12085802); Chlorophyll b (PubChem CID: 11593175).

* Corresponding author.
E-mail address: nymanm@rpi.edu (M.C. Nyman).

https://doi.org/10.1016/j.focha.2022.100178
Received 30 October 2022; Received in revised form 16 December 2022; Accepted 27 December 2022

(*e.g.,* leafy greens) during cold storage and processing. Extraction and recovery of phytochemicals depend on the nature of the food matrix, solvent choice, and phase separation process (Paradisoet al., 2020). Acetone and hexane are widely used solvents for plant pigment analysis for polar and non-polar carotenoids due to high extraction efficiency and high stability of the pigments in these solvents (Amorim-Carrilho et al., 2014; Pocock et al., 2004; Saini & Keum, 2018). Previously, molar extinction coefficients (ε_λ) for different carotenoid and chlorophyll compounds in acetone solvent have been determined. The ε_λ (in L mol^{-1} cm^{-1}) for chlorophyll a and lutein in acetone were found to be 78,750 at 662.7 nm and 144,500 at 446 nm, respectively (Craft & Soares, 1992; Jeffrey & Humphrey, 1975) (see also Supplemental Material Table S.1). In diethyl ether, the ε_λ of chlorophyll a increases to 90,200 at 660.8 nm (Porra et al., 1989).

A quick, accurate, and low-cost analytical method for the assessment of bioactive leaf pigments, such as carotenoids and chlorophylls, would be beneficial for plant growers and consumers. Most commonly, reversed-phase high performance liquid chromatography (rp-HPLC) analysis is the method of choice for quantification of leaf pigments (Amorim-Carrilho et al., 2014; Ashenafi, 2022; Pocock et al., 2004). However, with the HPLC method, a high capital cost for instrumentation and a significant amount of mobile phase solvents are required.

Early work on the application of spectrophotometry for analysis of algae and plant samples focused on the determination of chlorophyll compounds (Arnon, 1949; Porra et al., 1989). Spectral characteristics of chlorophyll standards were determined in different solvent assays and used to build a system of equations. The solvents used in the Porra et al. (1989) method were 80% buffered aqueous acetone, methanol, and dimethylformamide.

More recently, Lichtenthaler and Buschmann (2001) developed a simplified UV-VIS spectrophotometric method for the determination of chlorophylls as well as total carotenoids in leaf extracts. The method has been widely applied for the analysis of the photosynthetic pigments in different plant samples (Chen et al., 2016; Naznin et al., 2019; Urschel & Pocock, 2018). However, this method cannot be used to determine the individual phytochemicals in a leafy green sample.

In one study, the accuracy of three UV–VIS spectrophotometry based methods for total carotenoid analysis, Hornero-Méndez and Minguez-Mosquera (2001) and Lichtenthaler and Buschmann (2001), and Method of Mean, was examined on 28 different fruits and vegetables (Biehler et al., 2010). In comparison to values found from the HPLC analysis, all three UV-VIS methods overestimated the total carotenoid content. The highest overestimation was found with Lichtenthaler and Buschmann's method (21% higher overall). For chlorophyll-rich samples, such as kale and spinach, significantly higher values were reported with the Lichtenthaler and Buschmann's method, potentially due to underestimation of chlorophyll's contribution to light absorption in a leafy green sample. Overall, the different spectrophotometric methods for carotenoid analysis including Lichtenthaler's were developed for total (sum) value determination based on an assumed extinction coefficient for carotenoids (Lichtenthaler, 1987; Rodriguez-Amaya & Kimura, 2004). However, the analytical technique has rarely been extended for the determination of individual carotenoid compounds in mixed samples, such as leaf green extracts. This is mainly due to the spectral overlap or structural similarity between many of these phytochemicals and, hence, the resulting in complexity during spectral deconvolution (Domenici et al., 2014; Thrane et al., 2015).

In this study, the absorption spectra of individual chlorophyll and carotenoid standards in four organic solvent systems (*e.g.,* 80:20 v/v acetone:DI water, 80:20 v/v methanol:DI water, 80:20 v/v acetonitrile: DI water, and 40:40:20 v/v/v acetone:methanol:DI water) were measured using a UV-VIS spectrophotometer. Spectral characteristics, such as the local maxima wavelength and absorption ratios, were determined from these measurements in order to develop a UV-VIS spectroscopic method for leafy greens in the future. Furthermore, the stability of pigment standards at 8 °C temperature was investigated for each selected solvent system. The selected solvents are low cost and have relatively low environmental toxicity (Ashenafi, 2022; Saini & Keum, 2018). It is hypothesized that the polar nature of pigment molecules will influence their solubility and stability in solvent systems. The findings from this study will be used to develop an analytical method for the simultaneous analysis of individual carotenoids and chlorophylls in leafy greens using UV–VIS spectrophotometry. Unlike previous methods (*e.g.,* Lichtenthaler & Buschmann (2001)), the proposed approach can provide the concentrations of the principal carotenoid compounds in plants.

Table 1
Solvent systems used in this study.

Solvent System	Volume fraction (%) Acetone	Methanol	Acetonitrile	DI Water
80:20 (v/v) ACE:DI	80	–	–	20
80:20 (v/v) MeOH:DI	–	80	–	20
80:20 (v/v) ACN:DI	–	–	80	20
40:40:20 (v/v/v) ACE:MeOH:DI	40	40	–	20

ACE = acetone; MeOH = methanol; ACN = acetonitrile; DI = deionized water.

2. Materials and methods

2.1. Chemicals

Primary-grade lutein and zeaxanthin standards with 98.6% purity were obtained from ChromaDex (Irvine, CA). Standards for neoxanthin (≥ 97%), violaxanthin (≥ 95%), chlorophyll *a* (≥ 85%), and chlorophyll *b* (≥ 90%) were supplied by Sigma-Aldrich (St. Louis, MO). The *β*-carotene standard (99.4%) was obtained from Santa Cruz Biotechnology (Dallas, TX). Meanwhile, HPLC-grade acetonitrile, acetone, and methanol (> 99%) were purchased from Fisher Scientific (Waltham, MA). Methyl *tert*-butyl ether (MTBE; 99.8%) and triethylamine (99.5%) were obtained from Acros Organics (Fair Lawn, NJ) and Fisher Scientific (Waltham, MA), respectively. Chemicals were used as received without further purification or modification. A stock solution of pigment standards was prepared in acetone and stored at −80 °C until analysis.

2.2. Solvent systems

Four solvent systems were selected for pigment analysis based on previous work by Lichtenthaler and Buschmann (2001) and Porra et al. (1989). The selected solvent systems investigated were (*v/v*) 80:20 acetone:DI water (ACE:DI), (*v/v*) 80:20 methanol:DI water (MeOH:DI), (*v/v*) 80:20 acetonitrile:DI water (ACN:DI), and (*v/v/v*) 40:40:20 acetone:methanol:DI water (ACE:MeOH:DI) (see Table 1). Individual pigment standards (*e.g.,* lutein, zeaxanthin, neoxanthin, violaxanthin, chlorophyll *a,* chlorophyll *b,* and *β*-carotene) were dissolved in these solvent systems and analyzed. Duplicate dilutions were prepared for each standard. Pigment solutions were prepared in dim light at room temperature to minimize photodegradation and/or photoisomerization (Meléndez-Martínez et al., 2022). All the solvent systems do not appreciably absorb light in the visible region and, thus, did not interfere with absorption measurements.

2.3. UV-VIS absorption measurement

The light absorption of selected pigment compounds and leaf extracts was measured with a double beam UV-VIS spectrophotometer (UV-1800, Shimadzu Instruments, Kyoto, Japan). A quartz cuvette (1-cm path length) was used for the sample analysis. Triplicate absorbance measurements of visible light (400 – 700 nm) were performed for each pigment standard. The spectral resolution of the instrument was set to 1 nm. Recorded spectra were saved as SPC files and converted to word

processing files using UVProbe PC software (Shimadzu Instruments, Kyoto, Japan). Measurements were taken from the lowest to highest compound standard concentration in increasing order using appropriate solvents as blanks (see Table S.1). Normalized absorption plots of pigments were found by dividing the measured absorbance values at a specific wavelength by the absorbance at the λ_{max} for each pigment compound investigated (see Table S.1).

2.4. HPLC analysis

Plant pigments (carotenoid and chlorophyll compounds) were analyzed with a Prominence-*i* high-performance liquid chromatograph with a PDA detector (LC-2030 3D, Shimadzu Scientific Instruments, Kyoto, Japan). The HPLC method consisted of a YMC C-30 Carotenoid column (4.6 mm x 250 mm x 5 μm, YMC America, Allentown, PA) and was used for the separation of lutein, violaxanthin, neoxanthin, chlorophyll *a*, and chlorophyll *b*. An isocratic elution with a mobile phase of 81:15:4 (*v/v/v*) MeOH: MTBE: DI water at a flow rate of 1 mL min^{-1} was used for HPLC analyses. The injection volume was 10 μL. Details of the method can be found in Ashenafi et al. (2022). The carotenoid, β-carotene, is a highly hydrophobic compound and exhibited strong interaction with the C-30 column (t_R > 1-hr). Hence, a different HPLC method was employed for β-carotene analysis. A Shimadzu Premier C-18 column (4.6 mm x 150 mm x 5 μm,) was used for analysis, while the mobile phase was 80:15:5 (v/v/v) ACN: MeOH: triethylamine. The flow rate and the injection volumes were 1 mL min^{-1} and 10 μL, respectively. The detection wavelength for β-carotene was 452 nm. For both HPLC methods, the oven temperature was maintained at 23 ˚C. Concentration of compounds were determined using calibration data from newly prepared external standards at the time of each analysis. Duplicate injections were performed for each sample and external standards, and average area values were used to determine concentrations. Calibration data for each pigment standard used in this study can be found in Table S.2.

2.5. Pigment stability study

Independent serial dilutions were performed to obtain seven concentrations of each pigment standard in the four different solvent mixtures. Duplicate dilutions were prepared for the compounds at each concentration. Solutions were poured into 35-mL amber borosilicate tubes with Teflon® lined screw caps and stored inside a laboratory refrigerator at 8 ˚C for 7 days. The concentration range for selected compounds can be found in Supplementary Materials.

Triplicate absorbance measurements of each compound were performed daily at selected wavelengths of maximum wavelength for each compound investigated with a UV-VIS spectrophotometer. These wavelengths were violaxanthin (λ_{det} = 442 nm), neoxanthin (λ_{det} = 438 nm), lutein (λ_{det} = 446 nm), zeaxanthin (λ_{det} = 452 nm), β-carotene (λ_{det} = 452 nm), chlorophyll *a* (λ_{det} = 663.5 nm), and chlorophyll *b* (λ_{det} = 650 nm). At the end of the 7 days, HPLC analysis of the final supernatant solution was performed. Pigment solutions were allowed to reach room temperature in a dark environment before each HPLC or UV-VIS measurement.

2.6. Pigment extraction from kale leaves

Kale leaves grown in a hydroponic unit were harvested and flash frozen in liquid nitrogen. Frozen leaves were powdered with a mortar and pestle and placed in Eppendorf tubes. For each solvent system, one mL of the extraction solvent listed in Table 1 was added into Eppendorf tubes. The tubes were vortexed and centrifuged to extract the pigments and separate the supernatant from the plant debris. Finally, 400 μL of the supernatant was drawn from each tube and mixed with 1600 μL of the same extraction solvent. The UV–VIS absorption spectra of the diluted leaf extract (no pre-filtration step) were measured and analyzed.

3. Results and discussion

3.1. Spectral characteristics

Depending on the solvent system compounds were dissolved in, different absorption characteristics including local maxima and spectral ratios were obtained (see Tables 2 and 3). The water content and polarity of solvents are known to influence the location of λ_{max} for chlorophylls and carotenoids (Lichtenthaler, 1987). The III/II ratio represents the ratio of abs (λ_{III} – $\lambda_{baseline}$) to abs (λ_{II} – $\lambda_{baseline}$). The first term refers to the absorbance height of the third peak (III) from the baseline between the second and third peaks. Similarly, the second term refers to the absorbance difference between the second peak (II) and the baseline between the second and third peaks. Violaxanthin exhibited nearly identical absorptivity for the second (II) and third (III) peaks in all solvent systems. For β-carotene and zeaxanthin, the absorptivity of the second peak was significantly larger than that of the third peak as indicated by the small III/II ratios (Tables 2 and 3).

For the different compounds investigated, it can be observed that individual local maxima shift to longer wavelengths in decreasing polarity from 80:20 (v/v) MeOH:DI to 80:20 (v/v) ACN:DI, and finally to 80:20 (v/v) ACE:DI solvent systems. The shift was relatively small (0.5 – 4 nm) and resulted in a decrease in solvent polarity moving from methanol to acetone (solvatochromic shift). Within the same solvent system, bathochromic shift or longer local maxima wavelength were associated with carotenoids that contain a higher number of conjugated double bonds in their chemical structure (such as zeaxanthin and β-carotene that have 11 conjugated double bonds (c.d.b)) (Meléndez-Martínez et al., 2007). Highly conjugated carotenoids absorb photons with longer wavelengths with higher molar absorptivity coefficient (ε) in the 400 – 500 nm range. Violaxanthin and neoxanthin each contain 9 c.d.b., while lutein has 10 c.d.b in its structure.

Normalized UV–VIS absorption plots for a kale-leaf extract solution and pigment standards of carotenoids and chlorophylls in each solvent system are presented in Figs. 1 and S.1 – S.3. For the carotenoid compounds (violaxanthin, neoxanthin, zeaxanthin, and β-carotene), three absorption peaks with high spectral overlap can be observed in the 400 – 500 nm wavelength region for all solvent systems investigated.

It can also be seen that the absorption spectrum of kale leaf extract solution contains spectral “fingerprints” of chlorophyll and carotenoid compounds (*i.e.,* high light absorption in the 600 – 700 nm (“chlorophyll” region and three characteristic maxima between 400 and 500 nm in the “carotenoid” region). Saponification was not performed during the extraction of pigments from kale leaves.

3.2. Pigment time study

The stability of pigment standards in the selected solvent systems was examined over 7 days at a storage temperature of 8 ˚C. Based on absorption data obtained from UV–VIS measurements, lutein, neoxanthin, chlorophyll *a*, and chlorophyll *b* were highly stable in all solvent systems during the study period (see Fig. 2). Violaxanthin and zeaxanthin were relatively stable in each solvent system except in 80:20 (v/v) MeOH:DI, where roughly 40 – 50% decrease was observed at the end of the 7-day study period. The compound, β-carotene, was highly unstable in 80:20 (v/v) MeOH:DI. In fact, small crystals were visible in the β-carotene and zeaxanthin containing samples for that solvent system. This crystallization is likely the cause of the significant data variation as evidenced in the large error bars shown in some of the data in Fig. 2. Acetone is a highly volatile solvent, and it may result in slightly higher pigment concentration than initial values ($A/A_0 > 1$) due to solvent loss. This may have occurred in the pigment samples from 80:20 (v/v) ACE:DI and 40:40:20 (v/v/v) ACE:MeOH:DI systems in the present work. At the end of the 7-day study, the final pigment concentration in the selected solvent systems was further analyzed using the HPLC. Based on the ini-

Table 2
Spectral characteristics of selected carotenoids in 80:20 (v/v) MeOH:DI and 80:20 (v/v) ACN:DI.

	80:20 (v/v) MeOH:DI			80:20 (v/v) ACN:DI		
Compound	Local maxima wavelength (nm)	Baseline (λ)*	III/II ratio	Local maxima wavelength (nm)	Baseline (λ)*	III/II ratio
Violaxanthin	418, 441, 470	458	0.97	417, 441, 471	458	1.0
Neoxanthin	413, 437, 465.5	453	0.94	414, 438, 467	454	0.94
Lutein	423, 446, 474	462	0.63	425, 448, 476	464	0.61
Zeaxanthin	426, 452.5, 479	469	0.34	428.5, 455, 482	471	0.20
β-Carotene	426, 453, 480	470	0.18	427, 455, 481	472	0.18

* The baseline is the wavelength minima between the second (II) and third (III) peaks.MeOH = methanol; ACN = acetonitrile; DI = deionized water.

Table 3
Spectral characteristics of selected carotenoids in 80:20 (v/v) ACE:DI and 40:40:20 (v/v/v) ACE:MeOH:DI.

	80:20 (v/v) ACE:DI			40:40:20 (v/v/v) ACE:MeOH:DI		
Compound	Local maxima wavelength (nm)	Baseline (λ)*	III/II ratio	Local maxima wavelength (nm)	Baseline (λ)*	III/II ratio
Violaxanthin	418, 443, 473	460	0.99	418, 442, 472	460	1.0
Neoxanthin	415, 440, 469	456	0.93	414, 439, 467	455	0.93
Lutein	426, 449, 477.5	465	0.59	425, 448, 476.5	464	0.62
Zeaxanthin	429, 456, 483	472	0.32	429, 454.5, 482	471	0.34
β-Carotene	428, 456, 482	473	0.17	427, 455, 481	471	0.18

* The baseline is the wavelength minima between the second (II) and third (III) peaks.MeOH = methanol; ACE = acetone; DI = deionized water.

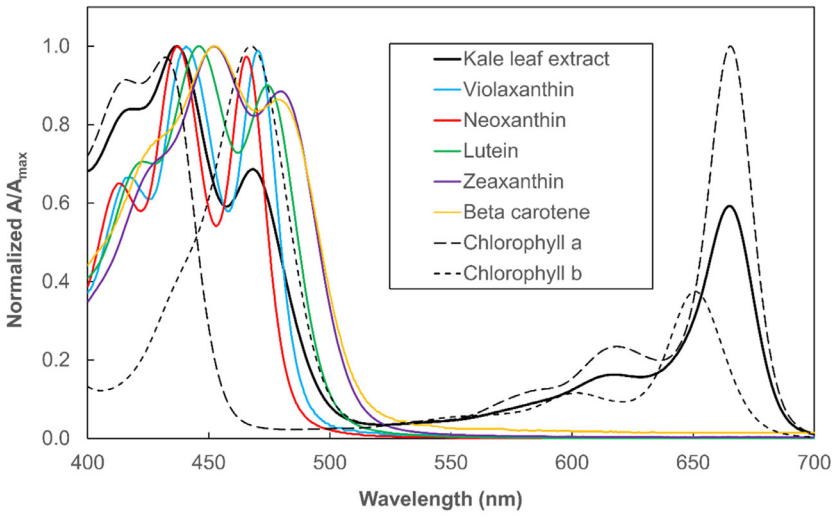

Fig. 1. Normalized UV–VIS absorption spectrum of carotenoids (solid lines with different colors), chlorophylls (broken lines), and leaf extract solution (solid black line) in 80:20 (v/v) MeOH:DI water system.

Table 4
Loss or disappearance of pigment standards in different solvent mixtures after 7-days of storage at 8 °C based on HPLC analysis.

Pigment	Solvent mixture			
	80:20 (v/v) MeOH:DI	80:20 (v/v) ACN:DI	80:20 (v/v) ACE:DI	40:40:20 (v/v/v) ACE:MeOH:DI
Lutein	< 10%	< 10%	< 5%	< 5%
Zeaxanthin	10 - 50%	< 25%	~ 0%	~ 0%
Violaxanthin	40 - 65%	< 5%	~ 0%	< 10%
Neoxanthin	< 10%	< 10%	~ 0%	~ 0%
β-Carotene	40 - 55%	80 - 86%	< 20%	16 - 90%
Chlorophyll *a*	< 10%	< 10%	< 10%	< 15%
Chlorophyll *b*	< 15%	< 15%	~ 0%	< 10%

tial and final concentration values, the percent loss was determined and presented in Table 4 below.

The HPLC data mostly confirm kinetic results from UV–VIS absorbance measurements (Fig. 2). An exception was found for β-carotene data, which exhibited a significant decline in 80:20 (v/v) MeOH:DI, 80:20 (v/v) ACN:DI, and 40:40:20 (v/v/v) ACE:MeOH:DI systems from the HPLC analysis. β-Carotene is a nonpolar carotenoid and has very low solubility in polar solvents, such as methanol and water (Paradisoet al., 2020; Zang et al., 1997). Lutein was relatively stable in each solvent system at the end of the 7-day study period, while zeaxanthin concentration was reduced by half at most in the 80:20 (v/v) MeOH:DI system.

Unknown peaks were observed in the chromatograms of neoxanthin, zeaxanthin, β-carotene, and chlorophyll *b* in the 80:20 (v/v) ACN:DI system. These peaks were potentially *Z*-isomers, which have different

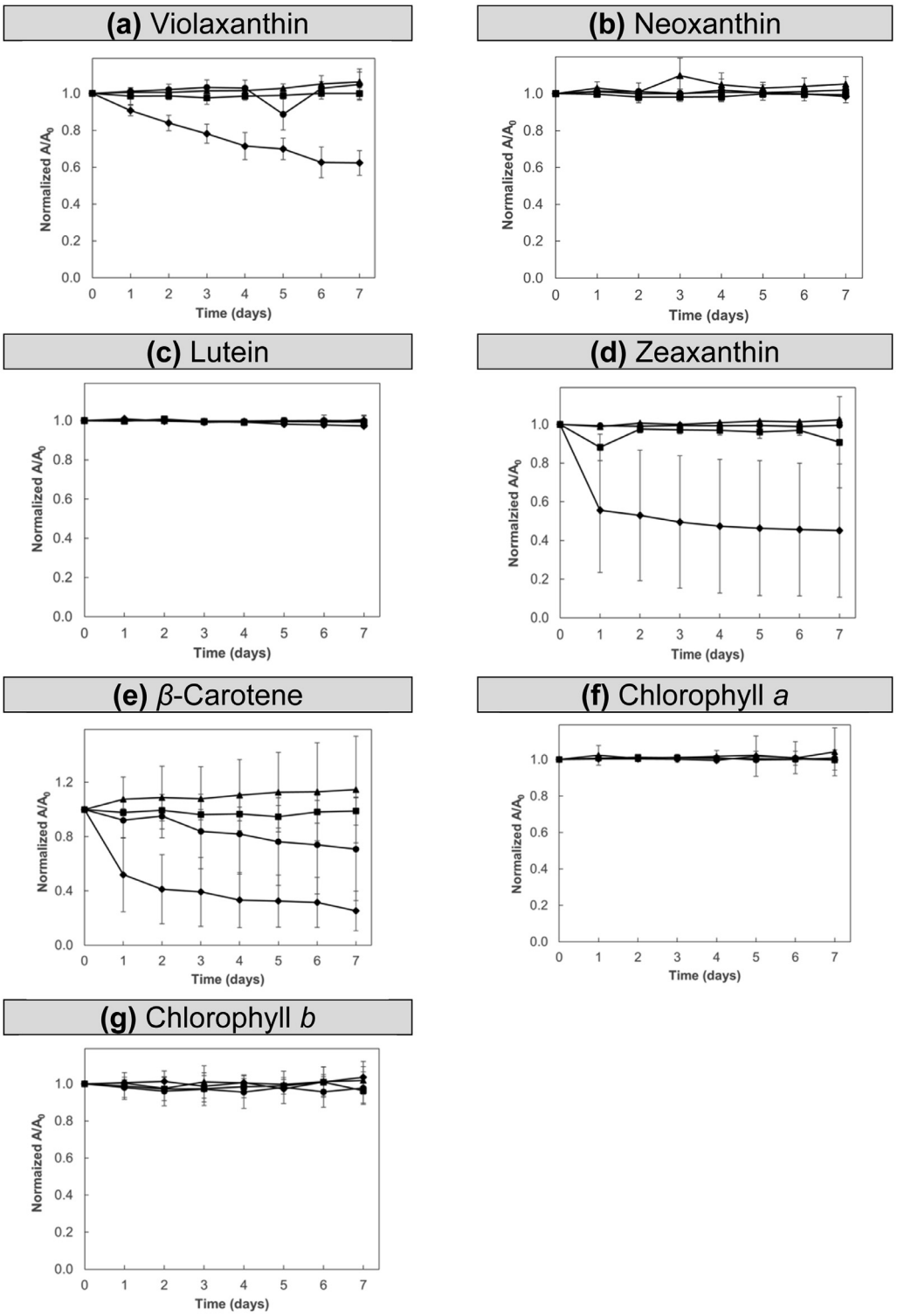

Fig. 2. Kinetics plots of pigment standards stored in amber borosilicate tubes at 8 °C in the solvents systems, 80:20 (v/v) MeOH:DI (♦), 80:20 (v/v) ACN:DI (■), 80:20 (v/v) ACE:DI (▲), and 40:40:20 (v/v/v) ACE:MeOH:DI (●). Error bars represent the mean ± standard deviation (SD) of normalized absorbance values (A/A_0). Based on UV–VIS measurements over 7 days.

retention times and light absorption characteristics than the all-*E* forms. In the present study, the stability of all-*E* geometric forms of selected carotenoids was only examined.

Xu et al. (2006) observed a 20% decrease in *β*-carotene and a 67% decrease in lycopene standard solutions stored at −20 °C after 70 days of storage in amber glass containers. Both compounds were dissolved in petroleum ether. The ether solvent was not considered in this study due to its incompatibility with the HPLC mobile phase and polar pigments such as lutein. Craft and Soares (1992) studied the stability of lutein and *β*-carotene in 18 organic solvents stored at ambient temperature. Based on absorbance measurements at λ_{max}, lutein was highly stable (≤10% decrease in concentration after 10 days) in acetone, methanol, or

acetonitrile, similar to the current findings. β-Carotene was also stable in these polar solvents with a maximum of 12% disappearance reported. In this work, β-carotene was only stable in the 80:20 (v/v) ACE:DI system.

Tang and Chen (2000) examined the stability of freeze-dried powder samples of lutein, α-carotene, and β-carotene under different storage temperatures with and without fluorescent lighting. First-order degradation kinetics ($r^2 > 0.95$) was observed for the analyzed carotenoids. The highest rate constant (fastest disappearance) was found with β-carotene under all treatment conditions. Furthermore, a significant isomerization of all-*E* to *Z*-isomers was detected in the samples stored at high temperatures (25 and 45 °C) and exposed to fluorescent light based on HPLC analysis.

Upon exposure to continuous white and UV-B light, a decrease in absorbance and formation of degradation products of chlorophyll *a* and *b* were observed in methanolic solutions (Petrović et al., 2017). A low degradation rate was observed at higher methanolic ratios, potentially due to the aggregation of chlorophyll molecules. In this study, limited change in chlorophyll concentration was found in each selected solvent system stored in darkness at low temperatures.

4. Conclusion

In this study, visible light absorption of chlorophyll and carotenoid compounds in organic solvents was measured with a standard UV–VIS spectrophotometer. The stability of pigment standards at a temperature of 8 °C was determined which showed varying degree of stability. Based on HPLC analysis, β-carotene was only stable in the 80:20 (v/v) ACE:DI solvent system upon storage at standard refrigerator temperature (4 to 8 °C). Significant loss of the compound (>40%) was observed in the other solvent systems. It would be useful to understand the isomerization and degradation pathways as well as the transformation products. This can be done by studying the unknown peaks and the compounds' mass spectra with HPLC analysis of *Z* standards and/or mass spectrometry analysis of the final supernatant solution. In addition to environmental factors such as temperature and light, the extraction efficiency of the solvent systems must be examined in real leaf samples. Findings from this study will be used to develop a mathematical model for plant pigment determination in leafy greens using UV–VIS spectroscopy.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Eyosias L. Ashenafi: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Marianne C. Nyman:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jacob T. Shelley:** Writing – review & editing. **Neil S. Mattson:** Writing – review & editing, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Acknowledgments

The work was supported by the National Science Foundation (NSF) Innovations at the Nexus of Food, Energy, and Water Systems (INFEWS) grant (Award # 1739163).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2022.100178.

References

Amorim-Carrilho, K. T., Cepeda, A., Fente, C., & Regal, P. (2014). Review of methods for analysis of carotenoids. *TrAC - Trends in Analytical Chemistry, 56*, 49–73. doi:10.1016/j.trac.2013.12.011.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta Vulgaris*. *Plant Physiology, 24*(1), 1–15. doi:10.1104/pp.24.1.1.

Ashenafi, E. L. (2022). *Optimization of controlled environment agriculture (CEA) for growing crops*. Rensselaer Polytechnic Institute Ph.D. thesis.

Ashenafi, E. L., Nyman, M. C., Holley, J. M., Mattson, N. S., & Rangarajan, A. (2022). Phenotypic plasticity and nutritional quality of three kale cultivars (Brassica oleracea L. var. acephala) under field, greenhouse, and growth chamber environments. *Environmental and Experimental Botany*, Article 104895. doi:10.1016/J.ENVEXPBOT.2022.104895.

Biehler, E., Mayer, F., Hoffmann, L., Krause, E., & Bohn, T. (2010). Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. *Journal of Food Science, 75*(1), C55–C61. doi:10.1111/j.1750-3841.2009.01417.x.

Chen, X. li, Xue, X. zhang, Guo, W. zhong, Wang, L. chun, & Qiao, X. jun (2016). Growth and nutritional properties of lettuce affected by mixed irradiation of white and supplemental light provided by light-emitting diode. *Scientia Horticulturae, 200*(2016), 111–118. doi:10.1016/j.scienta.2016.01.007.

Craft, N. E., & Soares, J. H. (1992). Relative solubility, stability, and absorptivity of lutein and β-Carotene in organic solvents. *Journal of Agricultural and Food Chemistry, 40*(3), 431–434. doi:10.1021/jf00015a013.

DellaPenna, D. (1999). *The photochemistry of carotenoids*. Kluwer Academic Publishers (C. R. J. Frank, H.A., Young, A., Britton, G (ed.)).

Domenici, V., Ancora, D., Cifelli, M., Serani, A., Veracini, C. A., & Zandomeneghi, M. (2014). Extraction of pigment information from near-UV Vis absorption spectra of extra virgin olive oils. *Journal of Agricultural and Food Chemistry, 62*(38), 9317–9325. doi:10.1021/jf503818k.

Hopkins, W. G., & Hüner, N. P. A. (2009). *Introduction to plant physiology* (4th ed.). John Willey & Sons. Inc.

Hornero-Méndez, D., & Minguez-Mosquera, M. I. (2001). Rapid spectrophotometric determination of red and yellow isochromic carotenoid fractions in paprika and red pepper oleoresins. *Journal of Agricultural and Food Chemistry, 49*(8), 3584–3588. doi:10.1021/jf010400l.

Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1, and c2 in higher plants, algae and natural phytoplankton. *Biochemie Und Physiologie Der Pflanzen, 167*(2), 191–194. doi:10.1016/s0015-3796(17)30778-3.

Joshi, D. R., & Adhikari, N. (2019). An overview on common organic solvents and their toxicity. *Journal of Pharmaceutical Research International, 28*(3), 1–18. doi:10.9734/jpri/2019/v28i330203.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*(C), 350–382. doi:10.1016/0076-6879(87)48036-1.

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*, (Supplement 1), 1–8 *F4.3.1-F4*. doi:10.1002/0471709085.ch21.

Lohr, M., & Wilhelm, C. (1999). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America, 96*(15), 8784–8789. doi:10.1073/pnas.96.15.8784.

McDonald, M. S. (2003). *Photobiology of higher plants*. Wiley.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2007). Relationship between the colour and the chemical structure of carotenoid pigments. *Food Chemistry, 101*(3), 1145–1150. doi:10.1016/j.foodchem.2006.03.015.

Meléndez-Martínez, A. J., Mandić, A. I., Bantis, F., Böhm, V., Borge, G. I. A., Brnčić, M., et al., (2022). A comprehensive review on carotenoids in foods and feeds: Status quo, applications, patents, and research needs. *Critical Reviews in Food Science and Nutrition, 62*(8), 1999–2049. doi:10.1080/10408398.2020.1867959.

Naznin, M. T., Lefsrud, M., Gravel, V., & Azad, M. O. K. (2019). Blue light added with red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. *Plants, 8*(4), 101–111. doi:10.3390/plants8040093.

Olives Barba, A. I., Cámara Hurtado, M., Sánchez Mata, M. C., Fernández Ruiz, V., & López Sáenz De Tejada, M. (2006). Application of a UV–Vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chemistry, 95*(2), 328–336. doi:10.1016/j.foodchem.2005.02.028.

Paradiso, V. M., Castellino, M., Renna, M., Santamaria, P., & Caponio, F. (2020). Setup of an extraction method for the analysis of carotenoids in microgreens. *Foods, 9*(4), 1–12 (Basel, Switzerland). doi:10.3390/foods9040459.

Petrović, S., Zvezdanović, J., & Marković, D. (2017). Chlorophyll degradation in aqueous mediums induced by light and UV-B irradiation: An UHPLC-ESI-MS study. *Radiation Physics and Chemistry, 141*(April), 8–16. doi:10.1016/j.radphyschem.2017.05.024.

Pocock, T., Król, M., & Huner, N. P. A. (2004). The determination and quantification of photosynthetic pigments by reverse phase high-performance liquid chromatography, thin-layer chromatography, and spectrophotometry. In *Photosynthesis research protocols* (pp. 137–148). Humana Press. doi:10.1385/1-59259-799-8:137.

Popova, A. V. (2017). Spectral characteristics and solubility of β-carotene and zeaxanthin in different solvents. *Comptes Rendus de L'Academie Bulgare Des Sciences, 70*(1), 53–60.

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 975*(3), 384–394.

Rodriguez-Amaya, D. B., & Kimura, M. (2004). *HarvestPlus handbook for carotenoid analysis*: 2. International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT).

Rodriguez-Amaya, D. B. (2016). *Food carotenoids: Chemistry, biology, and technology*. John Wiley & Sons, Ltd.

Saini, R. K., & Keum, Y. S. (2018). Carotenoid extraction methods: A review of recent developments. *Food Chemistry, 240*, 90–103. doi:10.1016/j.foodchem.2017.07.099.

Schieber, A., & Carle, R. (2005). Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. *Trends in Food Science and Technology, 16*(9), 416–422. doi:10.1016/j.tifs.2005.03.018.

Tang, Y. C., & Chen, B. H. (2000). Pigment change of freeze-dried carotenoid powder during storage. *Food Chemistry, 69*(1), 11–17. doi:10.1016/S0308-8146(99)00216-2.

Thrane, J. E., Kyle, M., Striebel, M., Haande, S., Grung, M., Rohrlack, T., et al., (2015). Spectrophotometric analysis of pigments: A critical assessment of a high-throughput method for analysis of algal pigment mixtures by spectral deconvolution. *PloS One, 10*(9), 1–24. doi:10.1371/journal.pone.0137645.

Tsao, R., & Deng, Z. (2004). Separation procedures for naturally occurring antioxidant phytochemicals. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 812*(1–2), 85–99. doi:10.1016/j.jchromb.2004.09.028.

Urschel, M. R., & Pocock, T. (2018). Remote detection of growth dynamics in red lettuce using a novel chlorophyll a fluorometer. *Agronomy, 8*(10). doi:10.3390/agronomy8100227.

Xu, F., Yuan, Q. P., & Dong, H. R. (2006). Determination of lycopene and β-carotene by high-performance liquid chromatography using sudan I as internal standard. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 838*(1), 44–49. doi:10.1016/j.jchromb.2006.04.004.

Zang, L. Y., Sommerburg, O., & Van Kuijk, F. J. G. M. (1997). Absorbance changes of carotenoids in different solvents. *Free Radical Biology and Medicine, 23*(7), 1086–1089. doi:10.1016/S0891-5849(97)00138-X.