Journal of Food Composition and Analysis 100 (2021) 103631

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

# Changes in compositions of galactolipids, triacylglycerols, and tocopherols of lettuce varieties (*Lactuca sativa L.*) with type, age, and light source

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ARTICLE INFO

*Keywords:*
Food lipids
Vegetables
Fat-soluble vitamins
Vitamin E
APCI-MS
APPI-MS

ABSTRACT

The compositions of molecular species of structural lipids and storage lipids of two types of lettuce leaves, romaine (green leaf) and Lolla Rossa (red leaf), are reported. Lettuce was harvested at two ages, three weeks (microgreens) and eight weeks (mature), and analyzed by liquid chromatography-mass spectrometry. Samples grown under natural light in a greenhouse were compared to those grown under grow lights. Compositions of molecular species of galactolipids (GALs), which are structural lipids, and triacylglycerols (TAGs) and diacylglycerols (DAGs), which are storage lipids, were examined relative to type (cultivar), age, and light source. GALs were most abundant, and the predominant molecular species were dilinolenoyl-monogalactosyldiacylglycerol (LnLn-MGDG) and dilinolenoyl-digalactosyldiacylglycerol (LnLn-DGDG), averaging 53.6 ± 1.4 % and 33.7 ± 2.3 % of galactolipids, respectively. TAGs containing linolenic acid (Ln) increased from three to eight weeks, especially LnLnL, LLLn, PLLn (P is palmitic acid and L is linoleic acid), and LnLnLn, with corresponding decreases in TAGs containing mostly linoleic acid, especially LLL, LLP, and LLO (O is oleic acid). α-Tocopherol started at similar levels in both varieties of greenhouse-grown lettuce, and it approximately tripled in Lolla Rossa, from 3.4 ± 0.3 μg/g to 9.9 ± 0.7 μg/g, by two LED lighting treatments.

## 1. Introduction

Lipids are fundamental components of all organic life, being referred to as one of the four "molecules of life", the four being proteins, carbohydrates, lipids, and nucleic acids (Purcell, 2018). Some plants produce large-scale amounts of lipids in the form of edible oils, like soybeans and olives, while others are known for their other characteristics, such as vitamins, minerals, and phytonutrients. The plants that makeup the majority of human consumption are fruits and vegetables. Although most of these are not remarkable for their lipid content, they do contain lipids, and these lipids can be an important part of the diet.

Lettuce varieties have been reported to be the most commonly consumed greens in the United States (Sharma et al., 2014; Kim et al., 2016). Loose leaf lettuces are reported to be nutritionally superior to head lettuces (Kim et al., 2016), and red-leafed lettuces higher in antioxidants than green-leafed lettuces (Buso, Bliss, 1988). For example, Crozier et al. (1997), reported 80 times more quercetin (a polyphenol) in Lollo Rossa than in a green "round lettuce".

Lettuce is mostly water by weight, with head lettuce typically being over 95 %. Even green leaf and red leaf lettuce are typically 94 % and 93 % water (Mou, 2009), respectively. Because of lettuce's mostly aqueous content, the nutrients, atoms, and molecules that have been most-studied in lettuce varieties are water-soluble metals, minerals, phytonutrients (anthocyanins, polyphenols, etc.), and other water-soluble components. Changes in these water-soluble nutrients and components have been examined with respect to lettuce type (leaf vs. head (Kim et al., 2016)), light source (e.g., greenhouse vs. artificial lighting), organic vs. conventional farming, age (microgreens (Xiao et al., 2012) vs. mature), and other variables. Recently, the phytonutrients in the same lettuces as used in our current study, but different samples, were reported analyzed for water-soluble components ($H_2O$/MeOH extract) by LC–MS, with processing through metabolomics software and proprietary flavanol identification software, FlavonQ (Sun et al., 2018). It is not surprising that the majority of attention has been given to the water-soluble components of this water-rich vegetable. However, lettuce also contains lipids, and these are part of the diet, so it is important to understand them, as well as the water-soluble components.

The lipid components from lettuce that have been most studied are the fat-soluble vitamins (FSVs) (Chun et al., 2006; Szymańska, Kruk,

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https://doi.org/10.1016/j.jfca.2020.103631
Received 27 September 2019; Received in revised form 2 July 2020; Accepted 21 August 2020
Available online 11 December 2020
0889-1575/Published by Elsevier Inc.

2008; Samuoliene et al., 2012) and carotenoids (Kim et al., 2018), though the fatty acid (FA) compositions of some varieties have been reported (Kim et al., 2016). Unfortunately, the FA composition does not distinguish FAs coming from different lipid classes, so there is no identification, and no quantification of different lipid classes, such as triacylglycerols (TAGs), diacylglycerols (DAGs), and galactolipids (GALs). Because of the lack of data on lettuce lipids, there is also no information regarding the intact individual lipid molecular species within any of these and other classes. Therefore, no changes in lipid molecular species have been reported with respect to the variables mentioned above (light, age, etc.) that have been used to probe changes in the water-soluble component compositions. There is one report of the TAG composition of lettuce seeds (El-Mallah, El-Shami, 2012), but not of the lettuce leaf. Thus, there is a paucity of data on the lipid composition of America's most-consumed leafy green vegetable. This report contributes to start to fill in the existing gap in knowledge of lettuce lipids.

Recent studies suggest that microgreens – seedlings of vegetables and herbs typically harvested 2–4 weeks after planting at the emergence of their first pair of true leaves – are considerably more nutrient dense than their more mature counterparts (Xiao et al., 2012), specifically in ascorbic acid, carotenoids (β-carotene, violaxanthin, and lutein/zeaxanthin), phylloquinone, and tocopherols (α- and γ-tocopherol). Such results have given rise to the appearance of interest groups in social media, books on how to grow microgreens, seed and equipment kits for growing them, and prevalence of microgreens in many grocery stores, farmers' markets, and restaurants (Treadwell et al., 2010). The initial research findings on microgreen nutrients suggested that microgreens, which are easy to grow in containers, indoors or outdoors, and during all seasons, might provide a way for people worldwide to locally access a wide range of beneficial nutrients in fresh and flavorful produce that can be grown in limited space, in most locations, and by amateurs, children, the elderly, and various groups that are currently nutritionally and economically impoverished.

This study reports a comparison of the lipids of two lettuce varieties commonly found in local and online seed stores: Lollo Rossa (red loose leaf) and Parris Island Romaine (green romaine with a creamy white heart). We report the compositions of lipid extracts of whole leaf lettuces with three variables considered: 1) type – green (romaine) versus red (Lolla Rossa) lettuce leaf; 2) age – three weeks (microgreens) versus eight weeks (mature); and 3) light treatment – greenhouse versus artificial light (4 treatments, one fluorescent and three LED).

## 2. Materials and methods

### 2.1. Plant material

Packets of two varieties of lettuce seeds (red and green *Lactuca sativa* L.) were purchased from a local garden store: 1) Livingston Seed Company (Columbus, OH, USA) Lollo Rossa (L) loose leaf lettuce 2015 seeds and 2) Grow Organic (Grass Valley, CA, USA) Parris Island Romaine (R) lettuce 2015 seeds. To ensure the roots could sufficiently develop as plants matured, twenty 16.51 cm × 44.45 cm × 31.75 cm (length × width × height) growing boxes were built using untreated cedar wood, with untreated poplar wood making up the upper six inches of the sides. The bottom third of each box was filled with an even mixture of 0.42 kg dry coconut coir, 1.77 L water, 0.47 L dehydrated cow manure, and 3.54 L of Coast of Maine Premium Potting Soil. The rest of each box was filled up to 7.62 cm below the top rim with an even mixture of 0.47 L of water, 0.15 L Coast of Maine Soil, 0.71 L dehydrated manure, 0.059 L azomite, 0.14 L alfalfa meal, and 2.37 L Vermont Compost. On June 19, 2015, one gram of seeds was spread evenly across the surface of the soil mixture in each box and covered with 6.35 mm of Jiffy Seed Starting Mix, a sheet of moist unbleached paper towel, and a 25.4 cm × 50.8 cm plastic tray which hung over the edge to allow airflow. Each box contained one lettuce cultivar, either L or R.

The boxes were aligned side by side in groups of four, and each group was placed on a 109.22 cm × 53.34 cm × 5.08 cm plastic boot tray, alternating boxes with each cultivar (e.g., LRLR). Each group was placed on a wire shelf in a climatized growth chamber. Four days after planting, after most seeds sprouted in each box, the towels and covering trays were removed. There were four light treatments in addition to greenhouse-grown: 1) Envirogro (now Agrobrite) T5 Fluorescent (Hydrofarm, Inc., Petaluma, CA, USA), 2) Diamond Series XML350 LED (Illuminatum, Inc. Rogers, AR, USA), 3) Propagator 38 LED Clone Lights, and 4) Illumitex NeoSol NS LED (Illumitex, Inc., Austin, TX, USA). Each light was hung at its manufacturer's recommended distance above its four boxes: 1) 30.48 cm, 2) 50.80 cm, 3) 45.72 cm, and 4) 121.92 cm, respectively. A timer was used to set the lights on a 14 h photoperiod. A fifth group of four boxes were placed in a greenhouse to allow for comparison with L and R lettuces grown in indirect sunlight. Daily, one liter of water was poured into the bottom of each tray that held four growing boxes, and the soil was watered from above using a spray bottle to keep the soil moist.

Plants were harvested and measured every seven days, starting on Day 14 (week two) and ending on Day 63 (week nine), according to a harvest/measurement protocol. Every week, each growing box was photographed, and then approximately half of the plants in each box were harvested in a manner that evenly thinned the remaining plants. In week six, plants were harvested so as to leave the eight strongest plants in each box, approximately evenly spaced; with four left in week seven; and two in week eight. When plants were young, each was harvested by cutting it one centimeter above the soil surface. When plants became larger, to avoid possible regrowth, each was pulled out with its roots and then the stem was cut above the root for measurement. All plants harvested from each box were then stored fresh frozen at −80 °C.

### 2.2. Light spectrum and other data

The lights did not come with documentation of their wavelengths, but information was available from most manufacturers' websites. No additional information could be found regarding the Propagator 38 LED clone lights. Although no documentation of the Envirogro T5 fluorescent light spectrum could be found, T5 fluorescent lighting has a well-known spectrum, showing peaks at 542.4 nm (green), and 546.6 nm (green), 611.6 nm (yellow), etc. (https://en.wikipedia.org/wiki/Fluorescent_lamp and https://commons.wikimedia.org/wiki/File:Fluorescent_lighting_spectrum_peaks_labelled.gif. The website of Advanced LED Lights (Illiminatum, Inc.) (https://advancedledlights.com/products/led-grow-lights/diamond-series-xml-2−0-350-with-usa-made-10w-cree-xml-leds/), maker of the Diamond Series XML350 LED, listed wavelengths of 760 nm, 740 nm, 720 nm, 660 nm, 630 nm, 615 nm-480 nm, 460 nm, 440 nm, 415 nm, 380 nm. Illumitex product brochures showed the F3 LED spectrum (http://illumitex.com/dev/wp-content/uploads/2012/07/bro_NeoSolNS.pdf) and listed it in tabular form (http://www.bocahydro.com/downloads/Neosoldsspecs.pdf) as: Blue (400–499 nm) 22.4 ± 1.3 %; Green (500–599 nm) 13.4 ± 0.6 %, Red (600–699 nm) 63.9 ± 0.8 %; Far Red (700–780 nm) 0.4 ± 0.1 %, with maxima at ~449 nm (blue) and ~657 nm (red).

HOBOware data loggers were set up at five locations in every grow box to record temperature, relative humidity, and illuminance at the soil level every 15 min over the period of growth. Results were exported to Excel using HOBOware software. The average measurement of illuminance (lum/ft$^2$) is summarized in Table 1.

### 2.3. Chemicals and reagents

Optima™ grade Methanol (MeOH) Acetonitrile (ACN), and dichloromethane (DCM), and certified ACS grade KCl were purchased from Fisher Scientific (Pittsburgh, PA). HPLC grade chloroform (CF) and crystalline vitamin $D_3$ (cholecalciferol, # C1357) and $d_6$-α-tocopherol (#731234) were purchased from Sigma-Aldrich (St. Louis, MO). 200 mL of 25.03 μg/mL internal standard (IS) solution of vitamin $D_3$ was made

**Table 1**
Average illuminance per light treatment measured in five locations in each growing box, averaged over 14 hours, in lux = lumens/m$^2$.

| Light Treatment | Height | Average Illuminance |
|---|---|---|
| Greenhouse | | 6,319 ± 1,432 lux ($n$ = 18) (variable throughout the day) |
| T5 Fluorescent Envirogro (Light #1, L1) | 12" | 27,086 ± 3,720 lux ($n$ = 20) |
| Diamond Series XML350 LED (Light #2, L2) | 20" | 15,390 ± 8,537 lux ($n$ = 18) |
| Propagator 38 LED Clone Lights (Light #3, L3) | 18" | 10,288 ± 4,209 lux ($n$ = 19) |
| Illumitex NeoSol NS LED (Light #4, L4) | 48" | 4,999 ± 1,264 lux ($n$ = 19) |

in 50:50 DCM/MeOH. 200 mL of 25.00 μg/mL $d_6$-α-tocopherol internal standard was made in 50:50 DCM:MeOH. 2.0 mL of Vitamin $D_3$ IS solution was added prior to extraction as an extraction IS (EIS), while 2.0 mL of $d_6$-α-tocopherol was added to the reconstituted lipid extracts prior to analysis as an analytical IS (AIS).

### 2.4. Sample preparation

All samples were freeze dried using a Labconco FreeZone 2.5 L lyophilizer (Labconco, Kansas City, MO) over several days. The lyophilized material was then ground to a powder using a Krups 203−42 Electric Spice and Coffee Grinder (Solingen, Germany). Samples were then stored at −20 °C.

#### 2.4.1. Folch extraction

Samples were prepared using the extraction of Folch et al. (1957). An EIS of 2.00 mL of a 25.03 μg/mL solution of Vitamin $D_3$ in 50:50 DCM:MeOH was added to 0.25 g of ground lyophilized material from Week 3 and 8, to account for any losses during the extraction. The Folch extraction began with adding 5 mL of 1:1 CF:MeOH solution, vortexing, and mixing for 20 min using mini magnetic stir bars. The homogenate was then centrifuged for 2 min at 1000 rpm, and the lower liquid phase was removed, via syringe with cannula, to another sample tube. The process was repeated five more times using 2.5 mL of CF:MeOH solution, each time adding to the liquid phase tube. Next, this liquid phase tube was washed with 5 mL 0.9 % KCl solution. After vortexing for 30 s, the mixture was centrifuged and the green CF lower layer was drawn off and then dried with a constant stream of nitrogen gas. The residue was weighed, and then reconstituted to 25 mL with 1:1 DCM:MeOH which had been bubbled with Argon for 15 min to minimize dissolved oxygen. The sample solutions were kept at −10 °C.

### 2.5. Galactolipid (GAL), triacylglycerol (TAG), and diacylglycerol (DAG) identification and relative quantification

One-dimensional HPLC with detection by three mass spectrometers simultaneously is known as triple parallel mass spectrometry or LC1MS3 (Byrdwell, 2011). An Agilent 1200 HPLC system (Santa Clara, CA, USA) was used with a MeOH/ACN/DCM gradient on two Inertsil ODS-2 columns (4.6 mm x 25.0 cm, 5 μm) in series at 1.3 mL/min at 10 °C, with 20 μL injections. Complete details for the chromatographic separation and UV and fluorescence detection (FLD) are given in the Supporting Material.

GAL, TAG, and DAG chromatographic peaks were identified, confirmed, and quantified using three mass spectrometers from Thermo Scientific (San Jose, CA, USA): 1) atmospheric pressure photoionization

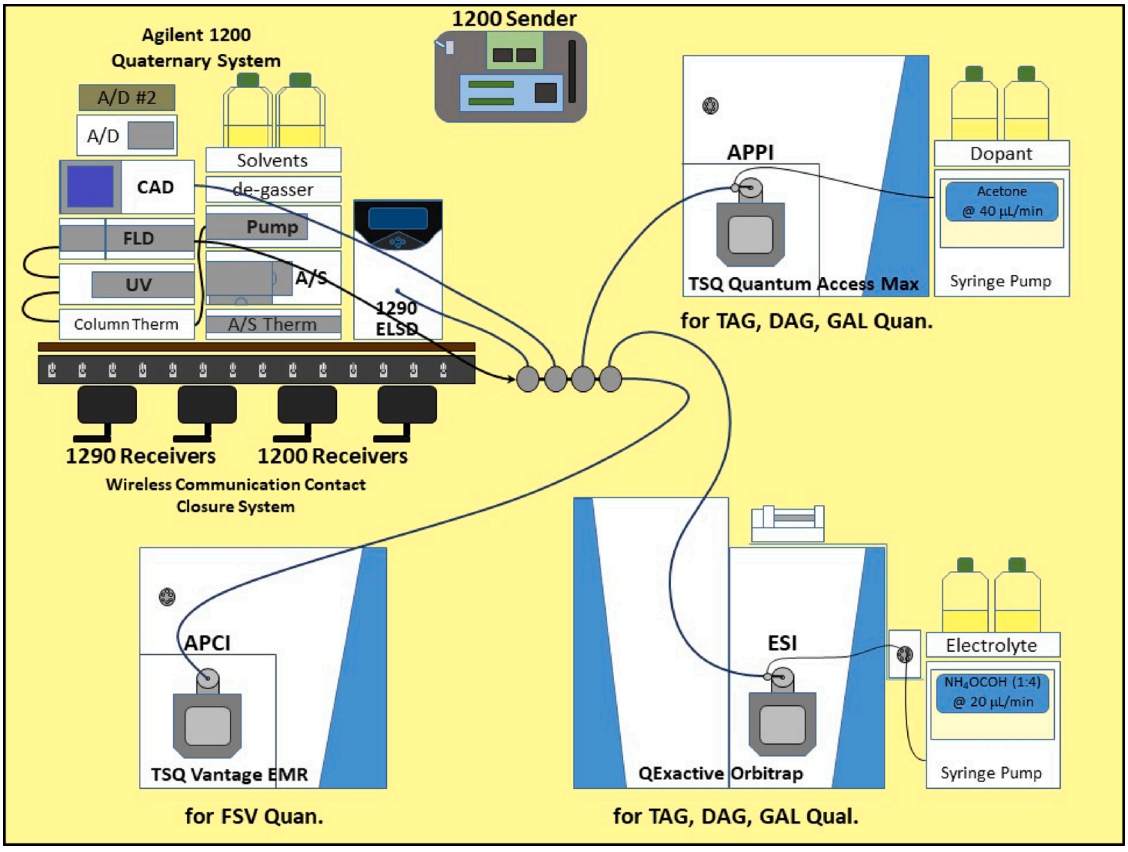


**Fig. 1.** Arrangement of instruments for LC1MS3. Agilent 1200 HPLC with ultraviolet detector (UV), fluorescence detector (FLD), evaporative light-scattering detector (ELSD), corona charged aerosol detector (CAD), atmospheric pressure photoionization (APPI) mass spectrometry (MS) on TSQ Quantum Access Max with acetone dopant at 40 μL/min, atmospheric pressure chemical ionization (APCI) MS on TSQ Vantage EMR, and electrospray ionization (ESI) MS on QExactive orbitrap with ammonium formate electrolyte at 20 μL/min. Instruments coordinated using wireless communication contact closure system.

(APPI) mass spectrometry (MS) on a tandem sector quadrupole (TSQ) Quantum Access Max mass spectrometer; 2) atmospheric pressure chemical ionization (APCI) MS on a TSQ Vantage EMR mass spectrometer; and 3) electrospray ionization (ESI) on a QExactive high-resolution, accurate-mass (HRAM) orbitrap mass spectrometer. The experimental arrangement is shown in Fig. 1. ESI-MS required 20 mM ammonium formate in methanol as electrolyte via syringe pump at 20 μL/min. APPI-MS required acetone as dopant, provided at 40 μL/min via syringe pump. Complete details for the ion source gas flows and heat settings, and scan ranges, speeds, resolutions, and other parameters for each of the three mass spectrometers are given in the Supporting Information.

The lipid chromatographic peaks were processed and manually integrated using the processing and "Quan Browser" functions in Xcalibur 2.2. The integrated peaks were used to calculate the percent relative abundance compositions of molecular species. Unlike fat-soluble vitamins (FSVs), which were quantified absolutely using calibration lines, GALs, TAGs, and DAGs were semi-quantified as percent relative compositions. Absolute quantification is not usually done for TAGs, etc., due to the large number of molecular species that would require calibration lines and the lack of commercially available standards for most (all but a few) molecular species.

APCI-MS was employed for FSV analysis, as previously reported (Byrdwell, 2017), because of its better response than APPI-MS. APPI-MS was applied for analysis of lipids as previously reported (Byrdwell, 2015), because of its better, more consistent signal-to-noise ratio over numerous chromatograms, due to its non-contact ionization process. ESI-HRAM-MS was used purely for qualitative analysis and peak identification and confirmation. Since galactolipids were found to be the largest proportion of lipids extracted, and thus the amounts of fatty acids (FAs) from GALs far exceeded the amounts of FAs from the TAGs, our conventional approach used for edible oils (Byrdwell et al., 2001; Byrdwell, 2017) of using the FA composition determined by GC-FID to make response factors (RFs) for TAGs could not be applied. Therefore, no response factors for TAGs were used. This introduced some small amount of error into the TAG composition, but based on past experience, the differences to RF-normalized results were expected to be small. The trends in the identities of the most abundant TAG molecular species are not expected to differ from RF-normalized results. Furthermore, relative changes in TAG molecular species between samples and trends therein would be similar with or without RFs, since RFs would be similar across all samples and would cancel each other out when considering differences.

### 2.6. Fat-soluble vitamin identification and quantification

Prior to LC–MS analysis, 25.0 mL sample flasks containing lettuce leaf extracts were placed under Argon to evaporate enough solvent to allow 2.0 mL of 25.0 μg/mL $d_6$-α-tocopherol AIS to be added, and the volume was made back to exactly 25.0 mL.

Generic multi-analyte calibration standard solutions containing vitamins A, D, E, and K, as well as retinol (vitamin A) palmitate and α-tocopherol (vitamin E) acetate at levels of 0.2 μg/mL, 0.4 μg/mL, 1.0 μg/mL, and 2.0 μg/mL with 1.25 μg/mL of $d_6$-α-tocopherol were analyzed, although only tocopherols were quantified for this report. Four calibration levels were used instead of the five levels reported elsewhere (Byrdwell, 2017) to reduce the sequence run time, due to the large number of runs necessary for this analysis. Sequences of runs for each of the five light treatments contained four calibration standards and four samples (red, green, 3 wk, 8 wk), each in triplicate, for 24 runs per sequence (12 × 54 min runs for FSV standards and 12 × 130 min runs for samples), requiring a minimum of 36.8 h instrument time to complete.

Peak areas were manually integrated using Xcalibur 2.2 on the TSQ Vantage EMR for selected ion monitoring (SIM) and selected reaction monitoring (SRM). For SIM, the following calculated ion masses were used (as listed in Supporting Material): 1) α-tocopherol $m/z$ 431.389; 2) $d_6$-α-tocopherol $m/z$ 437.427; 3) γ-tocopherol $m/z$ 416.365 + $m/z$ 417.373; 4) vitamin $D_3$ $m/z$ 385.347. For SRM, the following calculated transition masses were programmed into the method: 1) α-tocopherol $m/z$ 431.389 → $m/z$ 165.149; 2) $d_6$-α-tocopherol $m/z$ 437.427 → $m/z$ 171.167; 3) γ-tocopherol $m/z$ 417.373 → $m/z$ 151.133; 4) vitamin $D_3$ $m/z$ 385.347 → $m/z$ 367.337. These transitions were performed in time segments corresponding to a time window around each analyte. All parameters are given in detail in the Supporting Material.

### 2.7. Calculations

Microsoft Excel spreadsheets were used for all calculations, and the "linest()" function was used to determine calibration line equations. Samples had enough vitamin $D_3$ added to give an expected amount of vitamin $D_3$ of 2.0 μg/mL in the final solution. The calibration solutions contained vitamin $D_3$, allowing us to quantify the amount of vitamin $D_3$ EIS recovered in the samples. Therefore, all FSV amounts quantified using the AIS were scaled by the EIS using a factor of 2.0 μg/mL/(amount of Vit. $D_3$ determined, in μg/mL) to compensate for differences in extraction efficiency between samples, and divided by the extract sample weight to give results initially in μg/g dry weight (DW), as follows: Amt. FSV [μg/g DW] = Amt. Detd.[μg/mL] × 25 mL × (2 μg/mL / EIS Amt. Detd. [μg/mL]) × (1/Lyophilized Sample Wt.[g]). Finally, samples were converted to μg/g FW by multiplying by the factor g DW/g FW.

Calibration standards had 1.25 μg/mL $d_6$-α-tocopherol AIS. Samples had 2.0 mL of 25.00 μg/mL $d_6$-a-tocopherol AIS added prior to analysis, giving 2.0 μg/mL AIS, as described above. A larger relative amount of AIS was used in samples compared to standards to allow us to use an integer volume (2 mL) by glass volumetric pipet instead of using plastic pipet tips, which could be degraded and contaminate samples by the DCM/MeOH sample solvent. Normally, 100 mL sample solutions containing 5.0 mL of 25 μg/mL (= 1.25 μg/mL) AIS would be made, but due to limited sample amounts from the small extract weights, 25 mL solutions were made. Therefore, the areas of all AIS peaks in real samples were scaled by a factor of 1.25/2.00 before dividing the analyte peak areas by the AIS peak areas and using the calibration lines to quantify the ratios of analyte/AIS by the internal standard approach. The limits of detection (LODs) and limits of quantification (LOQs) for each sequence of runs were calculated as LOD = 3 x $\sigma_b/m$, LOQ = 10 x $\sigma_b/m$, where $\sigma_b$ is the standard deviation in the intercept and $m$ is the slope of the calibration line.

Principal component analysis was conducted using Unscrambler X version 10.3 (CAMO Software, Oslo, Norway).

### 2.8. Abbreviations

Fatty acids (FAs): P, palmitic acid, C16:0; Po, palmitoleic acid, C16:1; Ln, linolenic acid, C18:3; L, linoleic acid, C18:2; O, oleic acid, C18:1; S, stearic acid, C18:0; A, arachidic acid, 20:0; B, behenic acid, 22:0; Lg, lignoceric acid, 24:0. Galactolipids (GALs): SQDG, sulfoquinovosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol.

## 3. Results

### 3.1. Plant growth

Fig. 2 shows photographs of the two lettuce varieties at eight weeks, the mature age chosen for compositional analysis, under the greenhouse conditions (control) and four light treatments. The pictures show a noticeable difference in the size and color of the leaves. For example, under L1, L2, and L4 light treatments, Lollo Rossa lettuce leaves had much more red pigment than under Greenhouse and L3 conditions. The red pigment in fruits and vegetables is associated with polyphenolic

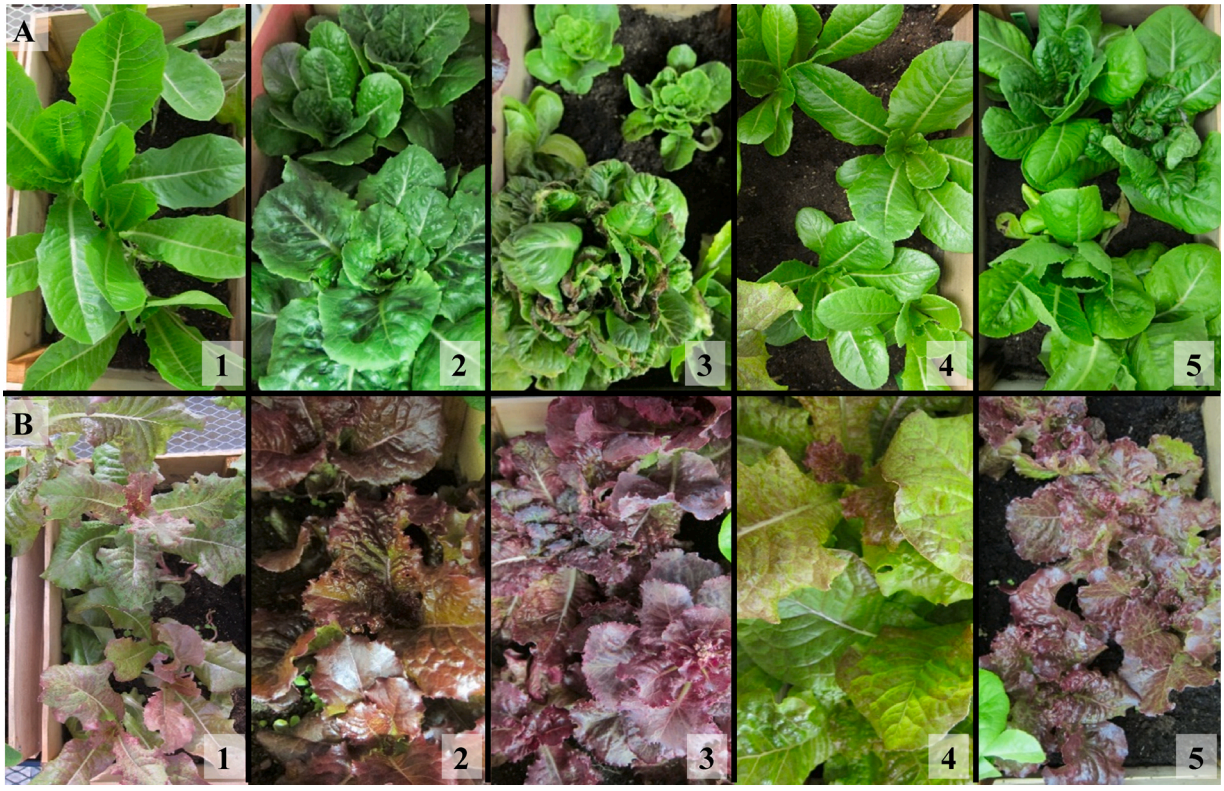


**Fig. 2.** Growth of A) Romaine Lettuce (Lactuca sativa L.) and B) Lolla Rossa lettuce at week 8, right before the harvest, under the five different light treatments. From left to right: 1) Greenhouse, 2) T5 Fluorescent Envirogro (L1), 3) Diamond Series XML350 LED (L2), 4) Propagator 38 LED Clone Lights (L3), and 5) Illumitex NeoSol NS (L4).

compounds, which have antioxidant properties (Gould et al., 2002).

The most pronounced head formation in Romaine lettuce occurred also under the L1, L2, and L4 light treatments. The Greenhouse light treatment consistently yielded considerably spindlier plants for both lettuce cultivars, probably due the shorter duration of higher illuminance per day. None of the measures of plant growth directly correlated

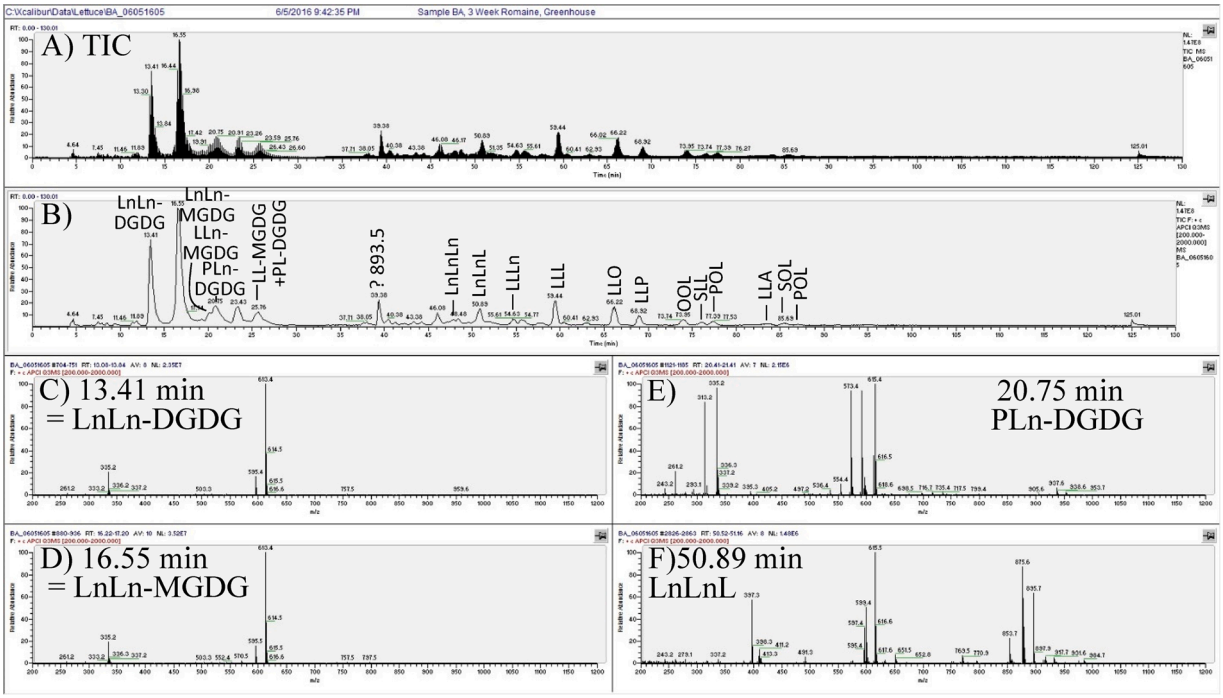


**Fig. 3.** APPI-MS chromatograms and mass spectra from lipid extract of greenhouse-grown 3 week old romaine lettuce (microgreens). A) Total ion current chromatogram (TIC); B) Full-scan chromatogram; C) average mass spectrum across LnLn-DGDG peak at 13.41 min; D) LnLn-MGDG peak at 16.55 min; E) PLn-DGDG peak at 20.75 min; F) LnLnL peak at 50.89 min.

with illuminance in this experiment, except for the distinctly spindlier plants under the more variable illuminance in the Greenhouse. The plants in Fig. 2A4, by L3, appear less corrugated, like greenhouse plants, and in Fig. 2B4 the Lolla Rossa show less color by L3 than by other light treatments.

### 3.2. Galactolipids (GALs)

Fig. 3 shows the APPI-MS total ion current chromatogram (TIC), full-scan filtered chromatogram, and mass spectra of three GALs and a TAG. Similarly, Fig. 4 exemplifies the ESI-HRAM-MS TIC, the full-scan filtered chromatogram, and mass spectra of the same molecular species as in Fig. 3. Fig. 3A and B show that the GALs were more abundant (larger peaks) and eluted earlier than the TAGs, hence results for GALs are presented first, since they were most abundant. APPI-MS was used for the quantification of the composition of GAL molecular species which are given in Table 2 and shown in Fig. 5, and ESI-HRAM-MS was used for confirmation of all lipid identities. All tables were sorted by percent relative composition averaged across all samples, largest to smallest. Due to the complexity of the table showing all samples, Table 2 shows results for only eight samples, 2 types x 2 treatments x 2 ages: 1) romaine and Lolla Rossa varieties; 2) greenhouse versus L2; 3) three-week and eight-week samples. L2 is an LED treatment that gave the sample (see Fig. 2A3, B3) with the highest level of α-tocopherol (see below). Supporting Table 12 gives the GAL compositions of all samples, with both types of lettuce under all light treatments for both ages, so 2 types x 5 treatments x 2 ages = 20 samples. All 20 samples are shown in Fig. 5.

The surprising takeaway from these results was their consistency, showing not much change between lettuce types and across ages. Principle component analysis (PCA), not shown, also indicated no informative clustering and no single or few factors that stood out as major trends in the GAL compositions across all types, treatments, or ages. The most abundant molecular species of the most abundant class of lipids were dilinolenoyl-monogalactosyldiacylglycerol (LnLn-MGDG) and dilinolenoyl-digalactosyldiacylglycerol (LnLn-DGDG), averaging 53.6 ± 1.4 % and 33.7 ± 2.3 % of galactolipids, respectively. These two LnLn-GALs comprised 87.3 % of the GALs on average.

Some GALs did show minor trends. PLn-DGDG (average 4.2 ± 0.8 % across all, Supporting Table 12) was slightly higher in romaine samples than in Lolla Rossa. Similarly, LLn-MGDG (average 3.5 ± 0.9 %) was slightly higher in romaine samples than in Lolla Rossa. LL-MGDG (average 1.2 ± 0.4 %) and LLn-DGDG (average 0.7 ± 0.3 %) were also slightly higher in romaine than in Lolla Rossa. The only GAL that showed a consistent increase from three weeks to eight weeks across all samples was PLn-SQDG (average 0.6 ± 0.1 %).

### 3.3. Triacylglycerols (TAGs)

The results for the TAGs were very different from the results for the GALs. There were distinct changes in the TAG composition with age, which can be seen in Table 3 and Supporting Table 13. Again, due to complexity, Table 3 show results for only greenhouse-grown (GG) and L2-grown samples. The complete table showing all 20 samples is given in Supporting Table 13, with those data depicted in Fig. 6.

There were distinct trends across all samples except one. The Lolla Rossa sample under lighting L3 that showed the lack of pigmentation (Fig. 2B4) also showed the least change in the TAG composition of all samples. For most samples, there was a substantial increase in several Ln-containing TAGs with corresponding decreases in L-containing TAGs from three to eight weeks. For instance, Table 3 and Fig. 6 show that LnLnL in GG romaine increased from 3.8%–18.9 %, LLLn increased from 4.7%–15.3 %, PLLn increased from 2.0%–10.6 %, and LnLnLn increased from 1.4%–12.0 %. These dramatic increases were accompanied by similarly dramatic decreases in LLL (23.5%–6.6 %), LLP (10.2%–6.5 %), LLO (20.3 % to 2.7 %), OOL (7.2 % to 1.0 %), POL (5.4 % to 1.5 %), and LLS (3.7 % to 0.6 %).

Fig. 7 shows the principal component analysis (PCA) plots of scores and influence across all samples. In Fig. 7A, samples colored in red and green are not well separated, indicating partial but not clear differentiation based on red-or green-leaf varieties, although red-leaf lettuce samples did appear to be higher in PC1 and PC2 together, than green-leaf samples. When grouped by age, Fig. 7B, the differences were clearer. The 3-week samples had lower values of PC1 than 8-week samples, with almost complete separation between groups. It was

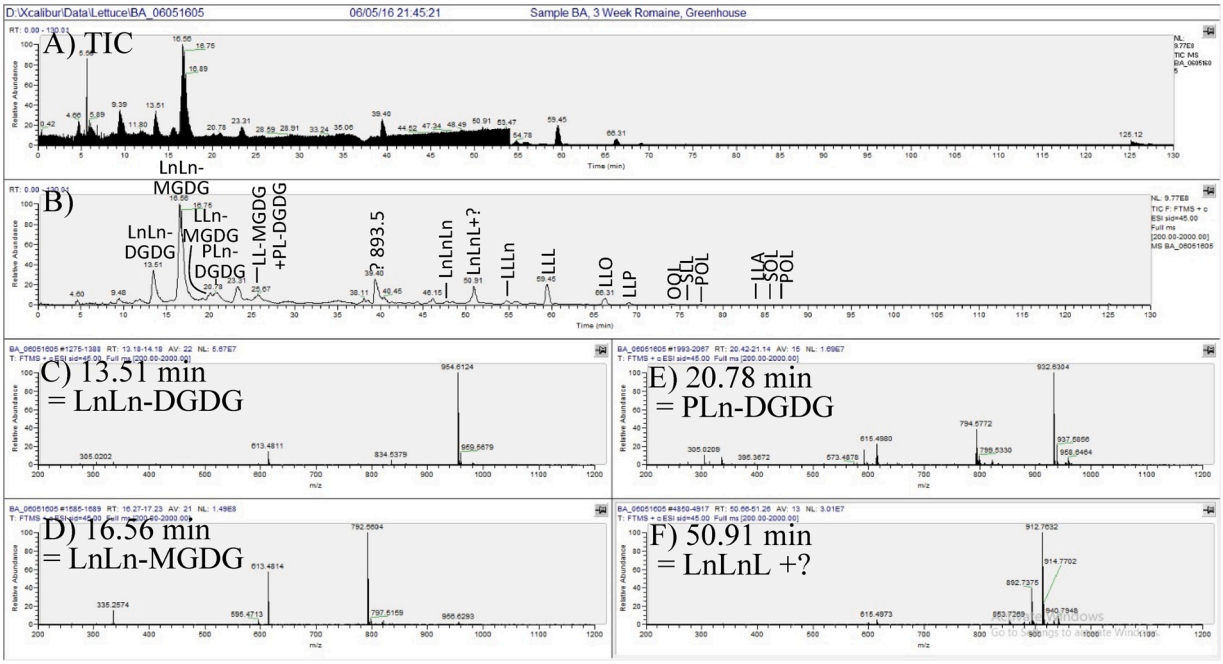


**Fig. 4.** ESI-MS chromatograms and mass spectra from lipid extract of greenhouse-grown 3 week old romaine lettuce (microgreens). A) Total ion current chromatogram (TIC); B) Full-scan chromatogram; C) average mass spectrum across LnLn-DGDG peak at 13.51 min; D) LnLn-MGDG peak at 16.56 min; E) PLn-DGDG peak at 20.78 min; F) LnLnL (+?) peak at 50.91 min.

**Table 2**
Galactolipid (GAL) percent relative compositions by HPLC-APPI-MS for greenhouse grown and L2-lighted red- and green-leaf lettuce lipid extracts. All runs in triplicate, $n = 3$.

| | Greenhouse | | | | L2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Romaine | | Lolla Rossa | | Romaine | | Lolla Rossa | |
| GAL | 3Wk | 8 Wk | 3 Wk | 8 Wk | 3 Wk | 8 Wk | 3 Wk | 8 Wk |
| LnLn-MGDG[a] | 53.4 ± 1.4 | 55.2 ± 1.8 | 52.9 ± 1.4 | 53.8 ± 0.9 | 55.7 ± 0.6 | 53.2 ± 0.2 | 53.2 ± 0.7 | 53.9 ± 1.2 |
| LnLn-DGDG | 27.5 ± 1.2 | 30.4 ± 1.2 | 32.5 ± 1.4 | 33.9 ± 0.7 | 31.7 ± 0.7 | 33.4 ± 0.1 | 37.5 ± 0.4 | 35.3 ± 1.3 |
| PLn-DGDG | 5.2 ± 0.4 | 5.4 ± 0.2 | 4.2 ± 0.1 | 4.6 ± 0.1 | 5.6 ± 0.2 | 4.8 ± 0.4 | 3.3 ± 0.2 | 3.8 ± 0.2 |
| LLn-MGDG | 5.7 ± 0.3 | 3.2 ± 0.2 | 4.1 ± 0.1 | 3.1 ± 0.1 | 2.9 ± 0.1 | 3.7 ± 0.1 | 2.4 ± 0.2 | 3.2 ± 0.1 |
| LL-MGDG | 2.6 ± 0.1 | 1.3 ± 0[b] | 1.6 ± 0 | 1.0 ± 0 | 1.1 ± 0.1 | 1.2 ± 0.1 | 0.8 ± 0 | 0.9 ± 0 |
| PL-DGDG | 1.3 ± 0.1 | 0.9 ± 0.1 | 1.3 ± 0 | 0.6 ± 0 | 0.6 ± 0 | 0.9 ± 0 | 0.5 ± 0 | 0.4 ± 0 |
| LLn-DGDG | 1.5 ± 0.1 | 1.0 ± 0.2 | 1.2 ± 0.1 | 0.7 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0 | 0.3 ± 0.1 |
| LL-DGDG | 1.1 ± 0 | 0.8 ± 0 | 1.0 ± 0.1 | 0.6 ± 0 | 0.6 ± 0 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.4 ± 0.1 |
| PLn-SQDG | 0.5 ± 0.1 | 0.6 ± 0 | 0.5 ± 0 | 0.7 ± 0 | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0 | 0.7 ± 0 |
| PLn-MGDG | 0.8 ± 0 | 0.6 ± 0 | 0.5 ± 0 | 0.5 ± 0 | 0.7 ± 0 | 0.8 ± 0 | 0.4 ± 0 | 0.6 ± 0.1 |
| LnLn-SQDG | 0.2 ± 0 | 0.3 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.3 ± 0 |
| LLn-SQDG | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| PL-MGDG | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0.1 ± 0.3 | 0.1 ± 0.1 | 0.2 ± 0.2 |
| **Sum** | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % |
| %AG/GAL | 37.4 ± 0.9 | 3.6 ± 0.1 | 4.8 ± 0.1 | 4.9 ± 0.2 | 12.5 ± 1.7 | 14.4 ± 2.1 | 3.4 ± 0.3 | 16.1 ± 1.9 |

[a] Abbreviations: P, palmitic acid, C16:0; Ln, linolenic acid, C18:3; L, linoleic acid, C18:2; O, oleic acid, C18:1. AG, acylglycerols (TAG+DAG); DGDG, digalactosyl diacylglycerol; GAL, galactolipids (MGDG+DGDG+SQDG); MGDG, monogalactosyl diacylglycerol; SQDG, sulfoquinovosyl diacylglycerol.
[b] 0 % represents <0.05 %.

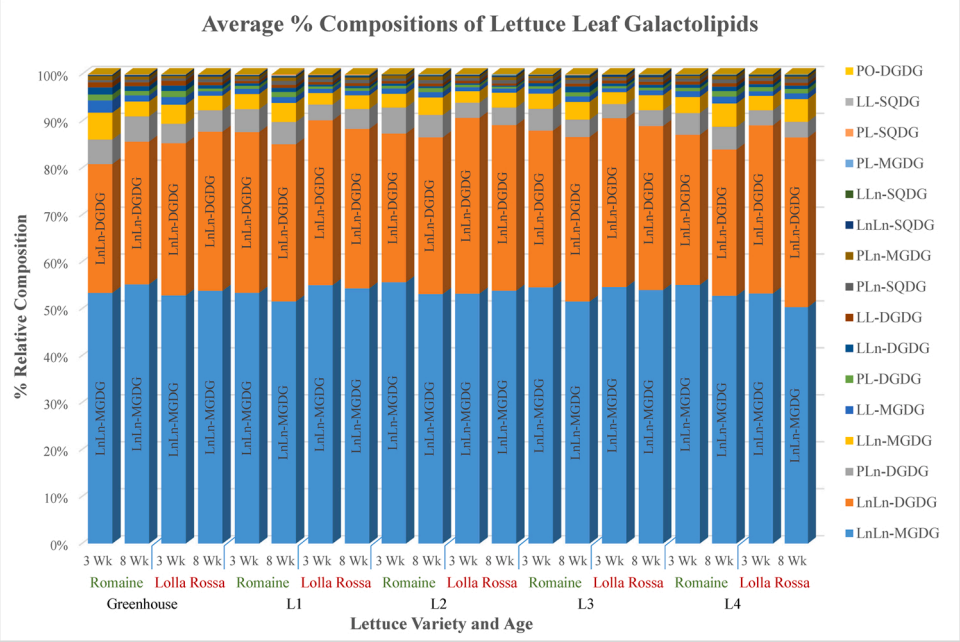


**Fig. 5.** Stacked bar graph of the 14 quantified galactolipids (GALs) in lettuce leaf extracts by variety (romaine and Lolla Rossa) and age (3 weeks and 8 weeks) from greenhouse-grown and light-treated (L1 to L4) lettuce plants. Abbreviations: P, palmitic acid, C16:0; Ln, linolenic acid, C18:3; L, linoleic acid, C18:2. SQDG, sulfoquinovosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol.

worth noting that the three-week GG romaine sample in Fig. 7A and B was somewhat of an outlier from the grouping of the other samples.

Finally, the loadings plot, Fig. 7C, shows the TAG molecular species responsible for most of the variance in the data, and clearly reflects the trend of Ln-containing species changing in the opposite direction of L-containing molecular species discussed above. The trends are so evident in Table 3 and Supporting Table 13 that it was not required to use PCA to clearly see the changes in TAG composition with age, but Fig. 7C does make very clear which TAG molecular species increased in PC1 (LnLnLn, LnLnL, LLLn, LnLnP, PLLn) and which ones decreased (LLL, LLO, OOL, LLP). Obviously, the shift toward more polyunsaturated TAGs would be related to increased desaturase enzyme activity in the growing plants. While all red- and green-leaf lettuce samples, except the Lolla Rossa under L3 lighting, exhibited the shift toward more unsaturation, there were some differences between the amounts of change between lettuce varieties. Lettuce that was GG, or grown under L1 lighting, or under L3 lighting showed greater changes in romaine versus Lolla Rossa (though both increased). Lettuce grown under the other two conditions, L2 lighting and L4 lighting, showed greater changes in the Lolla Rossa variety than in the romaine, though both changed substantially.

Thus, not only do these data provide the TAG compositions of lettuce lipid extracts, but they also show specific changes at the molecular species level that correlate to age and that vary with lettuce variety. Furthermore, because of the nature of the changes (from L-containing to Ln-containing TAGs) the data point to the specific mechanism of change within the plants (increased desaturase enzyme activity), thereby tying

**Table 3**
Triacylglycerol (TAG) percent relative compositions by HPLC-APPI-MS for greenhouse grown and L2-lighted red- and green-leaf lettuce lipid extracts. All runs in triplicate, $n = 3$.

| | Greenhouse | | | | L2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Romaine | | Lolla Rossa | | Romaine | | Lolla Rossa | |
| TAG | 3 Wk | 8 Wk | 3 Wk | 8 Wk | 3 Wk | 8 Wk | 3 Wk | 8 Wk |
| LnLnL[a] | 3.8 ± 0.1 | 18.9 ± 0.4 | 14.2 ± 0.4 | 24.7 ± 0.6 | 14.2 ± 2.1 | 17.0 ± 2.8 | 16.7 ± 2.4 | 23.7 ± 4.0 |
| LLLn | 4.7 ± 0.3 | 15.3 ± 0.1 | 16.7 ± 0.5 | 20.5 ± 0.2 | 13.9 ± 0.7 | 13.4 ± 1.1 | 16.6 ± 0.4 | 18.5 ± 1.5 |
| LLL | 23.5 ± 0.8 | 6.6 ± 0[b] | 11.4 ± 0.3 | 6.3 ± 0.1 | 10.9 ± 0.4 | 5.1 ± 0.3 | 9.2 ± 0.5 | 6.6 ± 0.6 |
| PLLn | 2.0 ± 0 | 10.6 ± 0.1 | 7.2 ± 0.2 | 8.3 ± 0.1 | 9.3 ± 0.4 | 14.0 ± 1.0 | 9.7 ± 0.4 | 11.1 ± 0.9 |
| LnLnLn | 1.4 ± 0.1 | 12.0 ± 0.2 | 6.4 ± 0.1 | 13.4 ± 0.4 | 5.8 ± 0.9 | 9.5 ± 1.6 | 7.7 ± 1.1 | 11.2 ± 1.3 |
| LLP | 10.2 ± 0.3 | 6.5 ± 0.1 | 7.3 ± 0.2 | 4.3 ± 0.3 | 7.8 ± 0.4 | 7.3 ± 0.4 | 8.0 ± 0.5 | 5.7 ± 0.6 |
| LLO | 20.3 ± 0.3 | 2.7 ± 0.1 | 4.9 ± 0.2 | 1.8 ± 0.1 | 7.2 ± 0.3 | 2.2 ± 0.2 | 2.6 ± 0.2 | 1.3 ± 0.2 |
| LnLnP | 0.6 ± 0 | 5.2 ± 0.2 | 2.8 ± 0.2 | 4.2 ± 0.2 | 3.9 ± 0.2 | 6.7 ± 0.5 | 4.0 ± 0.3 | 5.1 ± 0.6 |
| OLLn | 1.6 ± 0.1 | 3.3 ± 0.1 | 4.6 ± 0.2 | 3.2 ± 0.4 | 5.0 ± 0.4 | 3.1 ± 0.2 | 2.9 ± 0.2 | 2.5 ± 0.1 |
| POL | 5.4 ± 0.2 | 1.5 ± 0.1 | 2.1 ± 0.2 | 0.8 ± 0 | 2.5 ± 0 | 1.9 ± 0.1 | 1.7 ± 0.1 | 0.7 ± 0 |
| LnLnO | 0.4 ± 0 | 2.3 ± 0.3 | 2.2 ± 0.2 | 2.5 ± 0.2 | 1.9 ± 0.1 | 1.4 ± 0.1 | 1.8 ± 0.2 | 1.9 ± 0.2 |
| PPL | 0.7 ± 0.1 | 1.9 ± 0.1 | 1.2 ± 0.1 | 0.7 ± 0 | 1.5 ± 0.1 | 2.8 ± 0 | 1.8 ± 0.2 | 1.5 ± 0.1 |
| OOL | 7.2 ± 0.1 | 1.0 ± 0.1 | 1.8 ± 0.1 | 0.5 ± 0 | 2.5 ± 0.1 | 0.6 ± 0 | 1.3 ± 0.1 | 0.3 ± 0 |
| LLS | 3.7 ± 0 | 0.6 ± 0 | 0.9 ± 0 | 0.5 ± 0.1 | 1.4 ± 0.1 | 1.0 ± 0 | 0.8 ± 0 | 0.7 ± 0.1 |
| LnLS | 0.4 ± 0 | 0.9 ± 0.1 | 0.8 ± 0 | 0.9 ± 0.1 | 1.3 ± 0.1 | 1.7 ± 0.2 | 1.1 ± 0.1 | 1.2 ± 0.1 |
| LLPo | 0.5 ± 0 | 0.8 ± 0.1 | 1.3 ± 0 | 0.9 ± 0.1 | 0.9 ± 0 | 0.8 ± 0.1 | 0.9 ± 0 | 1.0 ± 0 |
| POLn | 0.3 ± 0 | 0.8 ± 0 | 0.8 ± 0 | 0.7 ± 0.1 | 0.8 ± 0.1 | 1.2 ± 0 | 0.8 ± 0 | 0.7 ± 0.1 |
| SOLn | 1.9 ± 0 | 0.6 ± 0.1 | 0.8 ± 0.1 | 0.3 ± 0 | 1.0 ± 0 | 0.8 ± 0 | 0.7 ± 0 | 0.3 ± 0 |
| PPLn | 0.1 ± 0 | 0.7 ± 0.1 | 0.3 ± 0 | 0.4 ± 0 | 0.8 ± 0 | 1.3 ± 0 | 0.7 ± 0.1 | 1.1 ± 0.1 |
| OOP | 1.3 ± 0.1 | 0.5 ± 0 | 0.8 ± 0 | 0.3 ± 0.1 | 0.4 ± 0 | 0.4 ± 0 | 1.6 ± 0.1 | 0.1 ± 0 |
| OOO | 1.4 ± 0 | 0.7 ± 0 | 0.7 ± 0 | 0.3 ± 0 | 0.4 ± 0.1 | 0.1 ± 0 | 1.5 ± 0.2 | 0.1 ± 0 |
| SLO | 2.2 ± 0 | 0.3 ± 0 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.7 ± 0 | 0.4 ± 0 | 0.4 ± 0 | 0.1 ± 0 |
| PoOL | 0.2 ± 0 | 0.6 ± 0 | 0.7 ± 0 | 0.5 ± 0.1 | 0.6 ± 0 | 0.9 ± 0.1 | 0.6 ± 0 | 0.5 ± 0 |
| SLP | 0.6 ± 0 | 0.4 ± 0 | 0.4 ± 0 | 0.2 ± 0 | 0.5 ± 0 | 0.8 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0 |
| POP | 0.3 ± 0 | 0.5 ± 0.1 | 1.1 ± 0.1 | 0.2 ± 0 | 0.2 ± 0 | 0.6 ± 0 | 0.7 ± 0 | 0.2 ± 0 |
| OOLn | 0.3 ± 0 | 0.4 ± 0 | 0.6 ± 0 | 0.3 ± 0 | 0.8 ± 0.1 | 0.3 ± 0 | 0.4 ± 0 | 0.2 ± 0 |
| MLLn | 0.2 ± 0 | 0.3 ± 0.1 | 0.4 ± 0 | 0.3 ± 0 | 0.4 ± 0 | 0.3 ± 0 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| PoPL | 0.2 ± 0 | 0.5 ± 0 | 0.6 ± 0 | 0.4 ± 0 | 0.4 ± 0 | 0.6 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 |
| LLnB | 0.1 ± 0 | 0.3 ± 0 | 0.4 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.5 ± 0.1 | 0.4 ± 0 | 0.3 ± 0 |
| MLL | 0.2 ± 0 | 0.2 ± 0 | 0.4 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0 |
| POS | 0.3 ± 0 | 0.3 ± 0.1 | 1.3 ± 0.1 | 0.3 ± 0 | 0.1 ± 0 | 0.4 ± 0 | 0.4 ± 0 | 0.1 ± 0 |
| LLA | 0.8 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.3 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.1 ± 0 |
| LLnA | 0.1 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.2 ± 0 | 0.2 ± 0 |
| LLB | 0.2 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.2 ± 0 | 0.2 ± 0 |
| OOS | 0.5 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.4 ± 0 | 0.1 ± 0 |
| MOL | 0.1 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.3 ± 0 | 0.4 ± 0 | 0.4 ± 0.1 | 0.4 ± 0 |
| MPL | 0.1 ± 0 | 0.2 ± 0 | 0.3 ± 0.1 | 0.2 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.3 ± 0 | 0.1 ± 0 |
| LOA | 0.6 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0 ± 0 |
| LLG | 0.3 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| LLnLg | 0 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.1 ± 0 |
| PLA | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.1 ± 0 |
| LLLg | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| PBL | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| SSO | 0.1 ± 0 | 0.2 ± 0 | 0.8 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| SSL | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| OLB | 0.1 ± 0 | 0.1 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 |
| OOA | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0.1 ± 0 | 0 ± 0 | 0.1 ± 0 | 0 ± 0 |
| OLG | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| OOPo | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| PLLg | 0 ± 0 | 0 ± 0 | 0 ± 0.1 | 0 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| POA | 0.1 ± 0 | 0.1 ± 0 | 0.3 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| MOLn | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| SLA | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 |
| PPP | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| OLLg | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.1 ± 0 | 0 ± 0 |
| PPS | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| POB | 0 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| SOA | 0 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| OOB | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| LLCe | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| POPo | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.1 ± 0.1 | 0 ± 0 |
| **Sum** | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % |

[a] Fatty acids: P, palmitic acid, C16:0; Po, palmitoleic acid, C16:1; Ln, linolenic acid, C18:3; L, linoleic acid, C18:2; O, oleic acid, C18:1; S, stearic acid, C18:0; A, arachic acid, 20:0; B, behenic acid, 22:0; Lg, lignoceric acid, 24:0.

[b] 0 % represents <0.05 %.

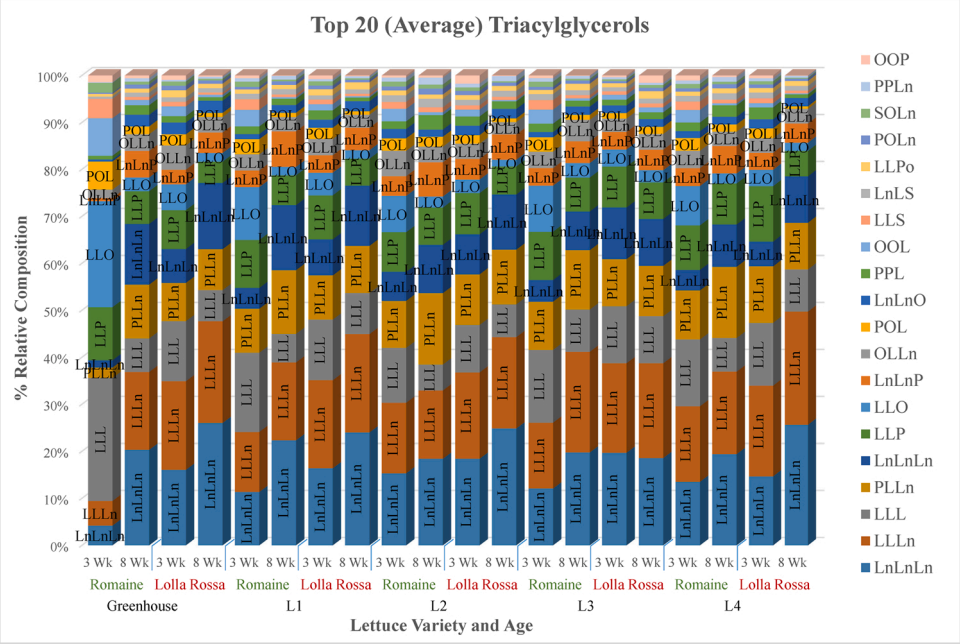


**Fig. 6.** Stacked bar graph of the 20 most abundant triacylglycerols (TAGs) in lettuce leaf extracts by variety (romaine and Lolla Rossa) and age (3 weeks and 8 weeks) from greenhouse-grown and L2 light-treated lettuce plants. Abbreviations: P, palmitic acid, C16:0; Ln, linolenic acid, C18:3; L, linoleic acid, C18:2.

together structure, function, and mechanism considerations regarding the TAGs in these plants. Finally, the dramatic differences in TAGs, compared to only small differences in GALs, show the very different responses to different pools of lettuce lipids with respect to age and environmental factors.

### 3.4. Diacylglycerols (DAGs)

DAGs gave two chromatographic peaks believed to correspond to different regioisomers, *sn*-1,3 versus *sn*-1,2 and *sn*-2,3 (*sn*, stereospecific numbering), which were quantified separately. The DAGs in Table 4 and Supporting Table 14 were present at low levels compared to TAGs, which were present at much lower levels than GALs, so caution should be applied to not over-interpret data based on small peaks. DAGs were present from a low of $4.3 \pm 0.1$%–$32.5 \pm 0.9$ % relative to TAGs. In general, PL (PL1 + PL2) was the predominant DAG, representing 55.3 % of DAGs on average, with LL (11.2 %), PLn (8.4 %), OL (8.2 %), SL (5.5 %), and OP (4.8 %) representing the bulk of DAGs. The most abundant DAG, PL2 ($41.7 \pm 8.5$ % average), did increase from three weeks to eight weeks in every sample, as did PLn2 ($6.3 \pm 2.0$ %). On the other hand, LL1 ($9.7 \pm 1.9$ %) decreased in every sample from three weeks to eight weeks, while OL2 ($5.4 \pm 2.5$ %) and OL1 ($2.9 \pm 3.1$ %) decreased in every sample except the Lolla Rossa sample treated by L3 lighting. Both OO1 and OO2 were higher in all three week romaine samples than in Lolla Rossa, and decreased in almost all samples from three weeks to eight weeks. Thus, there were some trends for DAGs, but these were less pronounced than the trends in TAGs.

### 3.5. Identification and quantification of fat-soluble vitamins

Fig. 8 shows typical SRM and SIM chromatograms for an eight week romaine sample extract (under L1 conditions) and the SRM and SIM calibration lines (CLs) from the standards used to quantify those peaks. The visual S/N for the SRM chromatogram is obviously higher than that for the SIM chromatogram. SRM is more specific than SIM because SRM involves a precursor → product transition by MS/MS, whereas SIM does not. SIM of α-tocopherol produces signal from any ion at $m/z$ 431.4, which is why SRM is considered more reliable for quantification. The CL by SRM shows less spread in the points at each calibration level and a higher coefficient of determination ($r^2$), with $r^2 = 0.9979$ by SRM and $r^2 = 0.9911$ by SIM. Nevertheless, we report the results by both SIM and SRM for comparison and contrast, and these agree well for most samples. Furthermore, having agreeing measurements by different ionization types on two different mass spectrometers increases the trustworthiness of results, especially those that have wider confidence intervals.

Fig. 9 shows the content of α-tocopherol for the two lettuce types at two ages under five different lighting conditions, while Supporting Fig. 1 shows the γ-tocopherol content for the same samples. The raw data for these graphs, including standard deviations, is given in Supporting Table 15. For GG samples, both varieties started with the same levels of α-tocopherol at three weeks (Fig. 9), and romaine was unchanged at eight weeks, while Lolla Rossa lettuce contained much more α-tocopherol (~3 times more) than romaine at eight weeks, confirmed by both methods of quantification (SRM and SIM). All lettuce grown under L1 and L2 conditions showed higher levels of α-tocopherol than GG lettuce at three weeks. The α-tocopherol in L1-grown Lolla Rossa lettuce increased slightly at eight weeks, but not as much as the GG. α-Tocopherol in both varieties of L2-grown lettuce started higher than in GG lettuce, and increased by 35–48 % in romaine, and increased by 160–175 % in Lolla Rossa, showing that this was by far the most effective light treatment of those tested, to increase α-tocopherol.

The lettuce grown under L3 lighting failed to thrive, which is evident in Fig. 2A4 & 2B4. The α-tocopherol concentration was higher in romaine by three weeks than GG romaine, but instead of rising, it fell to the same level as GG. L3 gave the worst performance for red-leaf lettuce. Samples started about the same as the GG samples, but fell to barely detectable. L3 produced the lowest amount of α-tocopherol at eight weeks, but due to the lack of same-instrument replicates, the data are not statistically rigorous, so are given for reference only. However, since we did have between-instrument replicates that matched closely, the reference values do have some confirmation.

The L4 treatment performed worse than (romaine) or equal to (Lolla Rossa) GG at three weeks, but at eight weeks it performed equal to (romaine) or better than (Lolla Rossa) GG. Although L4 produced the lowest α-tocopherol (of any sample) in romaine at three weeks, by eight weeks it produced more than GG, while for Lolla Rossa, it was about equal to GG.

Fig. 9 demonstrates the benefits of obtaining both SIM and SRM data.

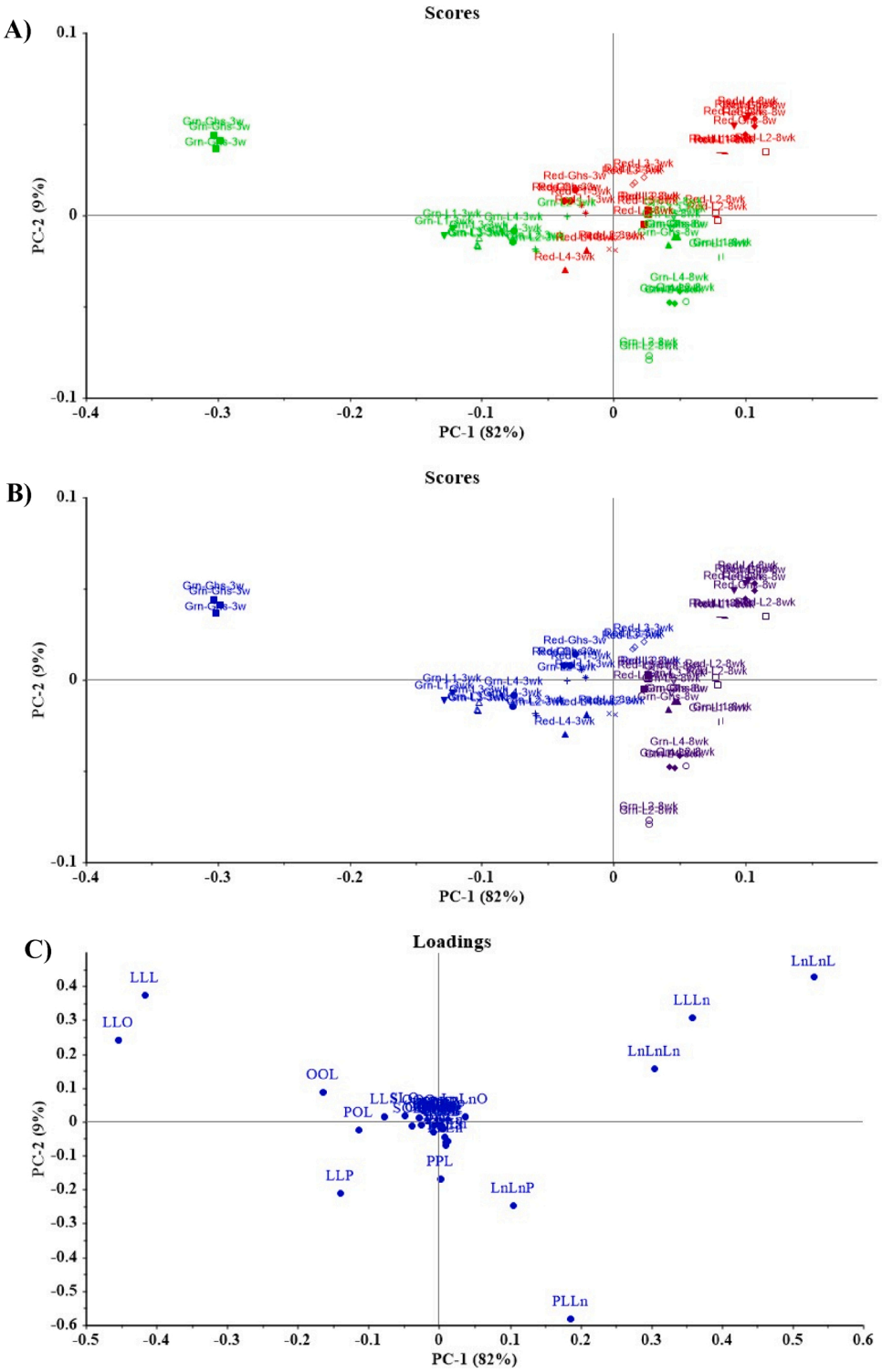


**Fig. 7.** Principal component analysis of triacylglycerols across all red- and green-leaf lettuce samples. See data in Table 2 and Supporting Table 1. A) Plot of PCA scores, showing sample groupings; B) Plot of PCA scores, showing age grouping; C) Plot of PCA loadings, showing the individual TAG molecular species most responsible for the variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

In most cases, the standard deviation (SD) in SIM results was lower than or equal to the SD in SRM results. In only one case for Lolla Rossa (L2, 3 wk), and two cases for romaine (L1, 8wk; L2, 8 wk) was the SD for SRM lower than for SIM. So, while SRM data are more authoritative, because SRM quantification comes from a specific precursor → product transition, the extra step of MS/MS with its inherent chemical noise, produces greater variability in most SRM results than in SIM results. Thus, Using the SIM data, with its lower SDs, gives confirmation of the quantification, while using SRM gave better identity confirmation with quantification.

The case with γ-tocopherol, Supporting Fig. 1, was quite different. The only consistent trend was that romaine contained more γ-tocopherol than Lolla Rossa, sometimes as much as almost seven times more (average SIM & SRM, GG green/red 3 wk = x6.85). In two cases of

**Table 4**
Diacylglycerol (DAG) percent relative compositions by HPLC-APPI-MS for greenhouse grown and L2-lighted red- and green-leaf lettuce lipid extracts. All runs in triplicate, $n = 3$.

| | Greenhouse | | | | L2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Romaine | | Lolla Rossa | | Romaine | | Lolla Rossa | |
| DAG | 3 Wk | 8 Wk | 3 Wk | 8 Wk | 3 Wk | 8 Wk | 3 Wk | 8 Wk |
| PL2[a] | 17.8 ± 0.9 | 45.6 ± 2.0 | 43.8 ± 1.1 | 46.2 ± 1.1 | 35.5 ± 0.7 | 45.1 ± 2.9 | 45.6 ± 1.4 | 49.4 ± 1.9 |
| PL1 | 11.1 ± 0.4 | 13.3 ± 0.6 | 15.7 ± 2.5 | 14.5 ± 0.6 | 12.2 ± 0.7 | 9.9 ± 1.3 | 18.0 ± 0.5 | 9.0 ± 1.2 |
| LL1 | 15.6 ± 2.0 | 9.8 ± 1.2 | 9.4 ± 1.2 | 8.4 ± 0.2 | 12.1 ± 0.5 | 9.3 ± 0.6 | 8.8 ± 0.5 | 8.4 ± 1.4 |
| PLn2 | 1.5 ± 0.1 | 7.1 ± 0.3 | 4.9 ± 0.4 | 8.2 ± 0.2 | 5.8 ± 0.5 | 9.2 ± 0.1 | 5.8 ± 0.3 | 10.2 ± 0.8 |
| OL2 | 13.5 ± 0.6 | 4.8 ± 0.2 | 6.5 ± 0.6 | 3.2 ± 0.2 | 7.9 ± 0.8 | 4.9 ± 0.5 | 4.7 ± 0.6 | 3.2 ± 0.1 |
| SL2 | 4.8 ± 0.1 | 2.7 ± 0.4 | 3.2 ± 0.3 | 3.6 ± 0.4 | 5.6 ± 0.6 | 4.9 ± 0.7 | 3.4 ± 0.3 | 4.0 ± 1.9 |
| OP2 | 4.1 ± 0.6 | 3.2 ± 0.2 | 4.9 ± 0.7 | 3.4 ± 0.2 | 3.9 ± 1.3 | 3.7 ± 0.3 | 3.5 ± 0.6 | 4.7 ± 0.6 |
| OL1 | 13.8 ± 1.3 | 1.7 ± 0.4 | 2.1 ± 0.2 | 1.8 ± 0.2 | 3.8 ± 0.5 | 1.3 ± 0.2 | 1.4 ± 0.2 | 1.1 ± 0.4 |
| PLn1 | 0.8 ± 0.1 | 3.0 ± 0.6 | 1.6 ± 0.4 | 3.4 ± 0.5 | 1.7 ± 0.4 | 3.1 ± 1.0 | 2.1 ± 0.2 | 2.2 ± 0.3 |
| SL1 | 3.5 ± 0.1 | 1.2 ± 0.4 | 1.3 ± 0.1 | 1.3 ± 0.3 | 3.3 ± 0.9 | 1.7 ± 0.5 | 1.4 ± 0.5 | 2.3 ± 1.5 |
| OLn1 | 1.1 ± 0.1 | 2.5 ± 0.2 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.5 ± 0.3 | 2.0 ± 0.2 | 1.0 ± 0[b] | 1.3 ± 0.1 |
| LL2 | 1.1 ± 0.1 | 2.5 ± 0.2 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.5 ± 0.3 | 2.0 ± 0.2 | 1.0 ± 0 | 1.3 ± 0.1 |
| OP1 | 3.1 ± 0.5 | 1.0 ± 0.2 | 1.3 ± 0.4 | 1.1 ± 0.4 | 2.1 ± 0.3 | 1.3 ± 0.5 | 1.4 ± 0.3 | 1.2 ± 0.1 |
| OO2 | 3.9 ± 0.2 | 0.8 ± 0.1 | 1.1 ± 0.1 | 0.9 ± 0.3 | 1.9 ± 0.6 | 1.0 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.1 |
| OO1 | 4.0 ± 0.3 | 0.4 ± 0.1 | 0.6 ± 0.1 | 0.4 ± 0.1 | 1.0 ± 0.2 | 0.5 ± 0.2 | 0.6 ± 0.1 | 0.5 ± 0.3 |
| OLn2 | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.2 ± 0 | 0.2 ± 0.1 | 0.3 ± 0.3 | 0.3 ± 0.1 | 0.2 ± 0.2 |
| **Sum** | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % | 100.0 % |
| DAG/TAG | 5.79 ± 0.26 % | 25.99 ± 1.11 % | 24.11 ± 1.11 % | 19.61 ± 1.41 % | 5.85 ± 1.19 % | 6.87 ± 1.14 % | 27.96 ± 3.97 % | 8.02 ± 1.03 % |

[a] Fatty acids: P, palmitic acid, C16:0; Po, palmitoleic acid, C16:1; Ln, linolenic acid, C18:3; L, linoleic acid, C18:2; O, oleic acid, C18:1.
[b] 0 % represents <0.05 %.

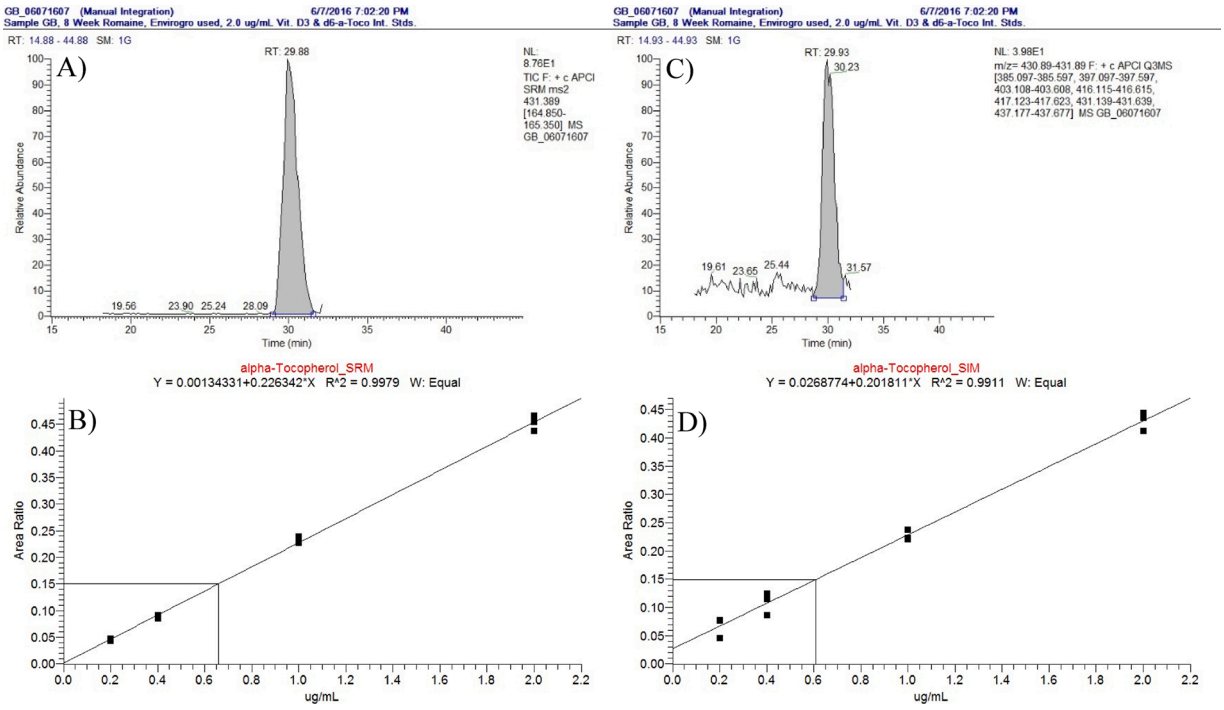


**Fig. 8.** Quantification of α-tocopherol by APCI-MS using $d_6$-α-tocopherol internal standard. A) Selected reaction monitoring (SRM) chromatogram of the transition $m/z$ 431.389 → 165.149 (calculated masses). B) Calibration line by SRM showing value from chromatogram in A). Coefficient of determination, $r^2 = 0.9979$. C) Selected ion monitoring (SIM) chromatogram of $m/z$ 431.389 (calculated). D) Calibration line by SIM showing value from chromatogram in C). Coefficient of determination, $r^2 = 0.9911$.

romaine (GG, L1), the γ-tocopherol decreased from three to eight weeks, in two cases (L2, L3) it approximately stayed the same, and in the final case it started negligible and increased. In Lolla Rossa, γ-tocopherol was generally low and stayed low, except in the case of the L2 treatment at eight weeks, which, at 5.5 ± 0.5–7.2 ± 0.2 μg/g FW averages for SIM & SRM, respectively, gave the highest γ-tocopherol content of the red-leaf lettuce samples, being an average of 6.4 ± 0.5 μg/g FW average for SIM and SRM. As was the case with α-tocopherol, the SIM results most often produced equal or lower SDs than SRM results, except in the case of L1 & L2 8 wk for romaine and L2 3 wk for Lolla Rossa. It is worth noting that the three-week GG romaine being the highest of all samples makes it somewhat of an outlier compared to the other samples. In results for Fig. 7A and B above, the three-week GG sample was mentioned to be somewhat of an outlier in the TAG composition, as well. This points to the need for more extraction replicates, in addition to the three analytical replicates already used.

The LODs and LOQs for all calibration sets is given in Supporting Table 16. The LOD and LOQ for α-tocopherol by SRM for the greenhouse

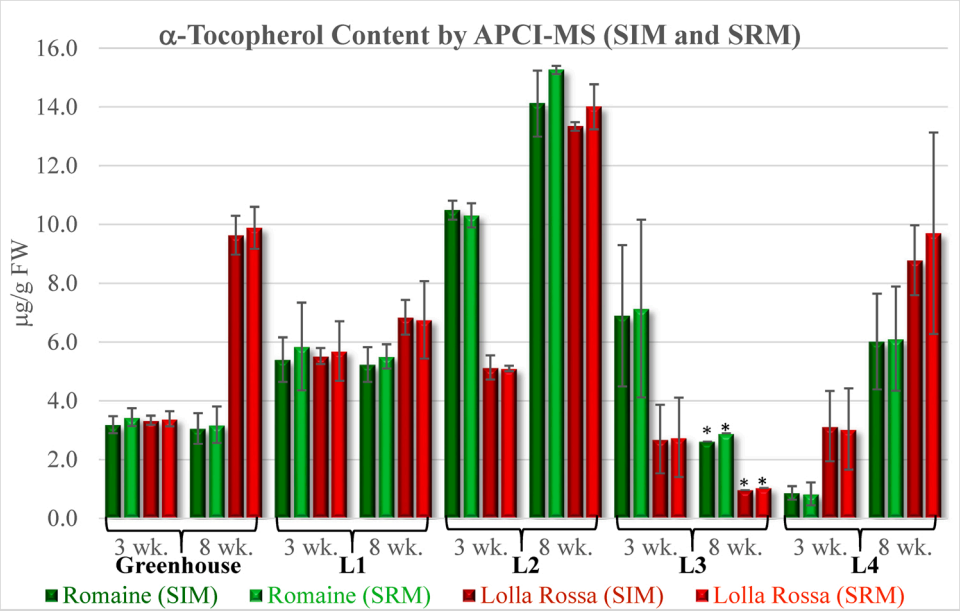


**Fig. 9.** α-Tocopherol content by APCI-MS SIM and SRM. Romaine lettuce colored in green and lolla rossa lettuce in red, each by greenhouse and four different light treatments. *Singlicate analysis, no statistical treatment possible; for reference only. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

samples was 0.3 µg/g FW and 1.0 µg/g FW, respectively, using the average lyophilization ratio. By SIM, the LOD and LOQ for α-tocopherol were 0.1 µg/g FW and 0.2 µg/g FW, respectively. The LOD and LOQ for γ- tocopherol by SRM for the greenhouse samples was 0.5 µg/g FW and 1.6 µg/g FW, respectively, and by SIM were 0.3 µg/g FW and 1.1 µg/g FW, respectively. In the case of the L3 γ-tocopherol results, the LOD and LOQ were so high as to be not usable for the low levels of γ-tocopherol in those samples. Thus, in addition to differences in growth, as seen in Fig. 2A4 & 2B4, the L3 samples also suffered from instrument failures that resulted in an unacceptably high LOD and LOQ. Finally, in all cases γ-tocopherol CLs had lower (poorer) coefficients of determination (CoDs), $r^2$, than α-tocopherol.

## 4. Discussion

### 4.1. Comparison to previous results

Numerous reports in the past have shown changes in tocopherols with different light treatments. However, most were targeted at and focused only on tocopherols, to the exclusion of other lipids. Specifically, we have not yet found reports of the compositions of other lipids in lettuce leaves including GALs, TAGs, and DAGs. Therefore, the dramatic changes in TAGs during growth from microgreens to more mature plants has not been reported. And the surprising consistency of GALs across different cultivars at different ages under different growing conditions has also not been previously reported.

A recent review demonstrates that the subjects of analysis of lettuce components have most been water-soluble phytonutrients, including anthocyanins and phenolics, although carotenoids have also been analyzed (Alrifai et al., 2019).

### 4.2. Triacylglycerols

We have not yet found any reports of the compositions of GALs, TAGs, or DAGs in lettuce, although a report by EL-Mallah and El-Shami (2012) did report the TAG composition of lettuce seed oil (LSO) from plants watered every 10 days (wet), 20 days (normal) or 30 days (dry). For normal LSO, the major TAGs were listed as LLL (24.2 %), LLO (20.0 %), LLP (10.5 %), and OOL (11.0 %), making up 65.7 % of the TAG composition. Our values for greenhouse-grown 3-week old lettuce (Table 2) for those same TAGs were LLL (23.5 %), LLO (20.3 %), LLP (10.2 %), and OOL (7.2 %), making up 61.2 % of the TAG composition. These values are surprisingly similar, given that one is from seed oil, while the other is from whole lettuce leaf. The major differences in TAG compositions in leaf versus LSO are the larger amounts of linolenic (Ln) acid-containing TAGs in whole lettuce. Molecular species such as LnLnLn, LnLnL, LnLnO, LnLnP, and LnLP that were not detected in LSO (El-Mallah, El-Shami, 2012) are present in substantial amounts in 3-week samples, and increase dramatically from three to eight weeks. These TAGs had values (3 wk → 8 wk) of LnLnLn (1.4 % → 12.0 %), LnLnL (3.8 % → 18.9 %), LnLnO (0.4 % → 2.3 %), LnLnP (0.6 % → 5.2 %), and LnLP (2.0 % → 10.6 %). Thus, as the lettuce aged, Ln-containing TAGs became the predominant TAGs. Therefore, it is logical that Ln-TAGs started at or near 0% in seeds, were present at comparatively low levels at three weeks, and increased from three weeks to eight weeks.

The dramatic changes in TAG composition with age and cultivar of lettuce, as well as different light treatments deserves more investigation. Even though they are present at lower levels (3–37 %) than GALs, they undergo much more dramatic changes due to age than GALs. Obviously, this points toward the need for investigation of the desaturase activity of lettuce plants (especially versus lettuce seeds). Overall, both TAGs and GALs in lettuce have very beneficial compositions of polyunsaturated FAs.

### 4.3. Tocopherols

The levels of tocopherols in lettuce have most often been reported in units of µg/g fresh weight (FW). Other units reported include mg/100 g FW and µg/g dry weight (DW) after lyophilization, as well as others. Since we lyophilized our samples, we initially prepared results in terms of DW, as shown in Supporting Figs. 2 and 3. Based on DW, it appeared that there were distinct trends showing substantial increases in α-tocopherol from three to eight weeks (Supporting Fig. 2) in four of five samples. There were corresponding decreases in γ-tocopherol from three to eight weeks (Supporting Fig. 6) in the same four of five samples. Furthermore, α-tocopherol was higher in Lolla Rossa than romaine for most samples, with converse results for γ-tocopherol. Thus, it appeared

that there were distinct trends that appeared in the data based on DW. Those trends disappeared when data were converted to a FW basis, even though no clear trends were evident in the DW/FW values (not shown). This points to a potential risk of using DW to determine results. Since DW is greatly affected by moisture, FSV concentrations based on μg FSV/g DW could be skewed by incomplete lyophilization or any other factors affecting dry versus fresh weights.

Based on FW, the values for α-tocopherol, Fig. 9, were surprisingly consistent across lettuce types and detection methods, especially three-week results for GG and L1 conditions, and eight week values for the sample treated under L2 conditions. α-Tocopherol did increase in Lolla Rossa under four of five light treatments, whereas romaine showed increases in only two of five cases. In the two cases of romaine (GG and L1) where α-tocopherol stayed the same, γ-tocopherol decreased, whereas in the one case where α-tocopherol was high in romaine and increased further from three to eight weeks (L2 conditions, Fig. 9), γ-tocopherol remained unchanged.

The list of literature values is given in Supporting Table 17. Our values are very much within the range of values reported in the literature for both romaine and Lolla Rossa varieties. Several authors reported values in units of mg/100 g FW, which were converted to μg/g FW. For instance, Chun et al. (2006) reported values ranging from 1.8 μg/g FW (converted from mg/100 g FW) to 7.4 μg/g FW for α- or γ-tocopherols in various lettuce varieties, with romaine having values of 5.5 μg/g FW and 3.6 μg/g FW for α- and γ-tocopherols, respectively. Cruz et al. (2014) reported values for butterhead lettuce of 17.3 μg/g and 14.0 μg/g FW for α- and γ-tocopherols, respectively, with no coffee grounds added to their soil. Santos et al. (2012) reported values of 10.0 μg/g and 6.0 μg/g FW for α-tocopherol in Ruby Red lettuce at 1 day and 10 days, respectively, with no α-tocopherol seen in green leaf lettuce. Cruz and Casal (2013) reported values of 1.47 μg/g and 5.11 μg/g FW (converted from μg/100 g FW) for α- and γ-tocopherols in green leaf lettuce, respectively, and values of 3.64 μg/g FW and 4.93 μg/g FW for α- and γ-tocopherols in red leaf lettuce, respectively.

One thorough article that nicely demonstrated the range of values of α-tocopherol and γ-tocopherol that can be obtained under different lighting conditions is the work by Samuoliene et al. (2013). They showed values that ranged from 0.31 μg/g FW to 2.4 μg/g FW for α-tocopherol, all the way to 11.60 μg/g FW with special light treatment. They showed a huge range for γ-tocopherol, from 6.54 μg/g FW to 9.12 μg/g FW, then up to 216.93 μg/g FW with special light treatment.

For perspective, the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) mentions that nuts, seeds, and vegetable oils are among the best sources of α-tocopherol (N.I.H. and O.D.S., 2019), with wheat germ oil providing 20.3 mg/serving, dry roasted sunflower seeds providing 7.4 mg/serving, and soybean oil giving 1.1 mg/serving. Green leafy vegetables also contain significant amounts, with spinach providing 0.6 mg/serving. Fig. 9 shows typical values from ~3 to 15 μg/g FW, which translates to 0.3–1.5 mg/100 g FW, meaning that lettuce, especially light-treated lettuce, constitutes a useful contribution to the daily intake of vitamin E.

In most cases, reported analyses were done using HPLC with fluorescence detection (FLD) in targeted approaches optimized for tocopherols. In contrast, we used a holistic analysis in which tocopherols and other FSV were detected and quantified, but a range of other lipid molecular species were also quantified. Fluorescence detection has the possibility to give an excellent signal-to-noise ratio and to achieve lower LODs and LOQs than APCI-MS. Thus, in our case some amount of sensitivity was sacrificed to be able to separate and quantify a wider range of lipid classes. Nevertheless, our values were very much in line with those reported using HPLC-FLD.

## 5. Conclusion

We provide here the first report of the percent relative compositions and changes of three classes of lipids of lettuce leaf extracts: 1) galactolipids, which are structural lipids, and 2) triacylglycerols and 3) diacylglycerols, which are storage lipids, in addition to showing tocopherol values that varied by age and light, as others have shown. This report clearly showed substantial changes in lipid compositions with increasing age, and lettuce variety, and substantial differences due to light treatment. Some dramatic changes with age were seen in the TAG compositions, in which greater changes were typically observed with age than were observed between varieties. On the other hand, the compositions of GALs were surprisingly consistent across all samples.

We have shown that the compositions of molecular species change from being linoleic acid dominant to being linolenic acid dominant, and identified the specific molecular species that changed the most. We also showed that the percent relative amounts of TAGs and DAGs relative to GALs changes. This is new information that deserves further investigation. Repetition of this study with many more samples would allow for better evaluation of the variability between extractions (extraction replicates), between individual plants, especially in the growth measurements, and allow for lower statistical variability. Of course, the difficulty of more replicates is the increased burden of data acquisition (instrument time and resources) and processing.

## Funding

This work was supported by the USDA Agricultural Research Service. Mention or use of specific products or brands do not represent or imply endorsement by the USDA.

## CRediT authorship contribution statement

**William C. Byrdwell:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Nicola Kubzdela:** Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization. **Robert Goldschmidt:** Methodology, Resources, Supervision.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103631.

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