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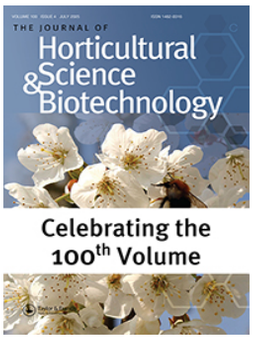

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The effect of supplementary LED illumination of Romaine lettuce on midribs pinking after harvest

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ABSTRACT

Pinking of midribs is a major postharvest issue in cut lettuce. Here, the effects of cultivar, light intensity and time of storage on pinking discolouration and related metabolites were elucidated. Two cultivars of Romance lettuce, Keona (fast pinking) and Icarus (slow pinking) were grown under four light intensities (L1 – L4: 1044, 578, 386 and 338 µmol.m −2.s −1 respectively); we determined their effects on pinking of leaf midribs, phenolic acids, soluble sugars and total ascorbic acid concentrations after eight days of cold storage. Differences in pinking index of the midribs of the two cultivars were only observed when the plants were grown in higher light intensities. All phenolic acids increased during storage and were highest at L1. Keona consistently contained higher concentrations of glucose, galactose and sucrose regardless of light intensity compared with Icarus after both 0 days and 8 days of storage. Principal component analysis (PCA) shows that coumaric acid, caffeic acid, and chlorogenic acid were positively correlated with the pinking index for both cultivars. The study revealed that pinking was reduced when the plants were grown at a low light intensity. Using low pinking cultivars offers a clear benefit in improving postharvest quality, especially when plants are grown under high light intensity.

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Oxidative pinking discolouration; *lactuca sativa*; light intensity; phenolic acids; soluble sugars

Introduction

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed fresh vegetables. 27 million tonnes are grown and utilised by eateries and food chains around the world in salads and sandwiches, or as a garnish, being appreciated for their neutral flavour, crisp texture, and green aesthetic. Lettuce is an annual crop, and it is one of the most economically important fresh vegetables cultivated mainly in moderate climates around the world (Kim et al., 2016).

Iceberg, Romaine, and loose-leaf lettuce are the three major types grown worldwide either for whole heads, minimally processed fresh-cut products, or as baby leaves to be used in salad mixes (Peng & Simko, 2023). Being a predominantly leafy vegetable, with a relatively reduced fibrous structure, it is susceptible to physical damage, which affects appearance and reduces consumer perception and acceptance. This is particularly relevant in cut and shredded preparations where damage is unavoidable. The most common damage in lettuce is enzymatic discolouration (browning or pinking) on cut surfaces, wilting, tissue deterioration, chlorophyll degradation, developmental senescence, russet spotting (due to exposure to ethylene), brown stain (caused by elevated levels of CO_2), and damages caused during cultivation by numerous biotic (such as pest and diseases) and abiotic factors (such as nutrient deficiency, water stress, salinity, high temperature, or mechanical injury during harvest, processing, and transportation (Moon et al., 2020; Peng & Simko, 2023).

Tissue discolouration of fresh lettuce is induced by wounding. Wounding triggers the synthesis of phenylalanine ammonia-lyase (PAL), the enzyme that is responsible for the formation of phenolic compounds through the phenylpropanoid pathway by conversion of phenylalanine to phenolic compounds including trans-cinnamic acid, p-coumaric acid, and ultimately to caffeic acid (Rawel & Rohn, 2010; Saltveit, 2018; Yoruk & Marshall, 2003). Newly formed and existing phenolics, which are initially compartmentalised inside the vacuoles of healthy cells, are released upon wounding and cell wall breakdown, consequently being exposed to polyphenol oxidase (PPO) in the cytoplasm (Degl'innocenti et al., 2005; Queiroz et al., 2008; Toivonen & Brummell, 2008). In unwounded cells, PPO is compartmentalised in plastids such as chloroplasts (Saltveit, 2018).

The enzymatic oxidation of phenolics happens in the presence of molecular O_2; a process that is mediated by PPO will produce quinones, which are further polymerised non-enzymatically (Chazarra

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et al., 1996; Martinez & Whitaker, 1995), to form brown or pink discolouration, which is suspected to be precipitation of melanin. The oxidation of phenolics to o-quinones by PPO can be reversed by antioxidants, such as ascorbic acid and L-cysteine, which reduce the o-quinones to their colourless o-diphenol precursors (Chen & Ho, 1997; Saltveit, 2018).

Light is an important abiotic factor that affects plant growth and development. As for other crops, differences in light intensities, spectrums and photoperiods affect the physico-chemical quality and shelf life of lettuce, and the effects vary depending on other aspects of growing conditions and crop variety (Dai et al., 2024; Landi et al., 2020; Song et al., 2020; Sutulienė et al., 2022). Increased light intensity promotes photosynthetic rate thus producing higher assimilate production. Carbohydrates are the backbone substances for the biosynthesis of other secondary metabolites. Among the secondary metabolites that are associated with oxidative discoloration of lettuce tissue are phenolic acids and ascorbic acid (Hunter et al., 2017; Saltveit, 2018).

Lettuce grown under high light intensity generally accumulates a high concentration of phenolic compounds (Gomez & Jimenez, 2020; Tomas-Barberan et al., 1997; Yan et al., 2019) and ascorbic acid (Gomez & Jimenez, 2020; Min et al., 2021; Ntagkas et al., 2019) compared to those grown at lower light intensity. From previous studies, phenolic acids in lettuce were observed to have a positive association with pinking discolouration. High light intensities increase the availability of soluble carbohydrates, especially glucose, and this contributes to the control of the ascorbic acid pool size as ascorbates in plants are regulated by the *D*-mannose/*L*-galactose pathway and ASC-related gene expressions (Ntagkas et al., 2018). Min et al. (2021) exposed loose-leaf lettuce to different light intensities (50, 210, and 470 µmol m^{-2} s^{-1}) for six days at the end of the production period with white-red LEDs and found that the high light intensities, though not notably higher than natural daylight, increased soluble carbohydrates and ascorbic acid at harvest. It is hypothesised that primary metabolites such as carbohydrates can play a role as precursors for secondary metabolites such as phenolic acids, though this relationship is uncertain. Min et al. (2021) also reported that ascorbic acid was found to correlate positively with carbohydrates at harvest and with the subsequent extension of shelf life, suggesting that the increased shelf life relies on the improved energy and antioxidant status of the crop at harvest. Tissues containing higher levels of ascorbic acid would be likely to have lower activity of PPO, thus possibly having low pinking incidence. With its antioxidant properties, ascorbic acid blocks the conversion of caffeic acid to caffeic acid-o-quinone or caffeic acid to chlorogenic acid, then to its o-quinones (Landi et al., 2013; Saltveit, 2018; Wen et al., 2021).

Manzocco et al. (2009) reported that light intensity around 44.5 µmol m^{-2} s^{-1} was found to be sufficient to inactivate the PPO activity in fresh-cut apples and this was associated with non-reversible structural changes of PPO, leading to enzyme inactivation. The increase in secondary metabolites and other phytochemicals in lettuce was also reported with increasing sunlight integrals but the results vary between cultivars (Woltering and Witkowska 2016; Z. Yan et al. 2019; Gomez and Jimenez 2020).

This paper is the first to report the consequences of adjusting pre-harvest light intensity on pinking incidence, phenolic acids, soluble sugars and ascorbic acid contents in two cultivars of Romaine lettuce with known differences in sensitivity to pinking during cold storage.

Materials and methods

Plant materials and growing conditions

Two cultivars of Romaine lettuce, Keona and Icarus (Rijk Zwaan, De Lier, Netherlands) were used. Keona is a fast pinking cultivar whereas Icarus is one of a Knox series, bred to be a slow pinking cultivar.

This study was conducted in a glasshouse at the University of Reading, UK. The seeds were sown in rockwool plugs (Grodan Industrieweg 15, 6045 JG, Roermond, Netherland) in plug trays of 77 cells, placed in a nutrient pool on 14 August 2023 and raised under natural sunlight. The seedlings were fertilised through capillary rise with a nutrient solution containing (mg L^{-1}): 100 N, 12 P, 128 K, 76 Ca, 14 Mg, 0.32 Fe, 0.32 Mn, 0.34 Zn, 0.07 B, 0.07 Cu and 0.017 Mo prepared using $Ca(NO_3)_2$ and a nutrient mix (Solufeed F, Solufeed Ltd., Chichester, West Sussex, UK) dissolved in tap water (electrical conductivity, EC = 0.50 mS cm^{-1}). The EC of the nutrient solution was 1.2 mS cm^{-1}.

The seedlings were transferred into a closed recirculated hydroponic growing system when they reached a 3-leaf stage (27 August 2023). The growing channels were made of 80 cm long PVC pipes (6.35 cm diam.). The distance between channels was 25 cm and the distance between planting holes within channels was 30 cm, giving a planting density of 13 plants m^{-2} (25 cm ×30 cm planting). In the hydroponic system, the plants were fed with a nutrient solution containing (mg L^{-1}) 152 N, 20 P, 208 K, 110 Ca, 22 Mg, 0.53 Fe, 0.53 Mn, 0.56 Zn, 0.12 B, 0.12 Cu and 0.028 Mo which was prepared using same source of fertilisers as the one used for the seedlings. To obtain the above-mentioned nutrient composition, 580 mg L^{-1} $Ca(NO_3)_2$ and 700 mg L^{-1} Solufeed F nutrient mix were mixed in tap water.

The EC and pH of solution were continuously monitored by an EC metre and a pH metre (Hanna Instruments Ltd, Leighton Buzzard, Bedfordshire, UK). The pH was maintained at 6.0 by injecting dilute nitric acid directly into the solution when necessary, by an acid injector (Hanna Instruments Ltd.). The EC of the solution was between 1.5–1.7 mS.cm^{-1}. The nutrient solutions in the 250 L tank were changed every 7 days.

Light treatments

The plants were grown under natural sunlight and supplemented with LED light source (8 Red: 1 Blue ratio, Tungsram^{TM}, Product ID: TUAS-GLIN) installed with 45° angle at one end of the whole growing system. A typical spectrum of the light emitted from the LED light is presented in Figure 1. The light treatments were arranged in a randomised complete block design with three replications. The light was turned-on for 16 h from 0600 to 2200 beginning at two weeks after transplanting (11 September 2023) to the end of the growing period (16 October 2023). Light intensities were estimated using an average of light emitted by the LED light source measured in total darkness (n = 5) + average of light levels in areas in the same glasshouse compartment that did not receive any supplementary light, measured at 1000–1030, 1230–1300 and at 1530 –1600 on 8 days during light treatment. The measurement was carried at 15 cm above the plant canopy using a light metre (SKP2000191–3668, Skye Instruments Ltd., Llandrindad, Wells, Powys, UK). The average light intensity levels received by plants in term of photosynthetically active radiation (PAR) for each light treatment were 1044 µmol m^{-2} s^{-1} (L1), 578 µmol m^{-2} s^{-1} (L2), 386 µmol m^{-2} s^{-1} (L3) and 338 µmol m^{-2} s^{-1} (L4) (Table 1). High light intensity (L1) was included as one of the treatments to emulate growing environment that receive high light intensity, so that the results generated from this study would have a wider range of practical applications. The average temperatures in the glasshouse during the growing period were 15 °C (night) and 33 °C (daytime).

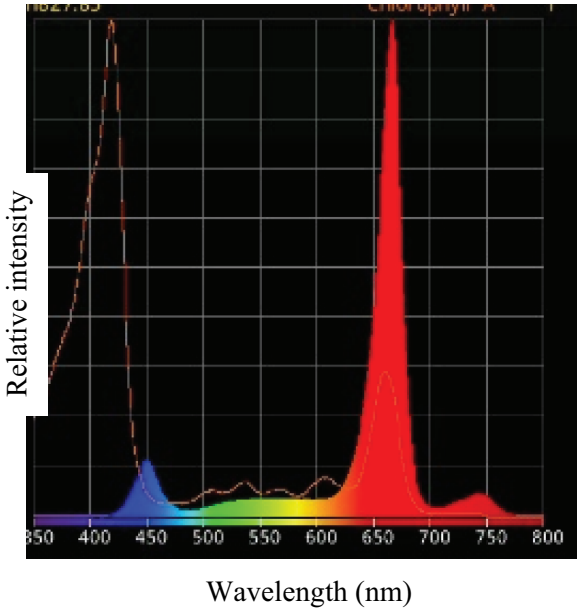

Figure 1. Light spectrums measured on a cloudy day at 10.11 (17 Sept 2023) using a hand-held spectral PAR metre at about 10 cm from LED light source.

Harvesting, sample preparation and cold storage

Two heads of lettuce for each experimental unit were harvested in the morning (9.30 to 11.30 am) on 9 October 2023 (43 days after transplanting) by cutting the plants at 2.5 cm above the media level. The plant material was immediately transported to the laboratory and stored at 5 °C before being processed for analysis the following day.

We observed that some leaves in plants planted in the first two rows near the light source were scorched and curled downward due to the effects of high light intensities. These samples were discarded from the experiment based on their lack of marketability.

During sample preparation, the two outer true leaves that were fully exposed to the environment were discarded as per typical producer practice to increase marketable aesthetics. Five leaves for each plant were removed from the core using a clean pair of scissors. Then, the midribs of the leaf were accurately separated from the leaf lamina using a knife that was cleaned after cutting of sample for each experimental unit to prevent cross-contamination and the midribs were used for analysis. The midribs were cut into 2.5 cm long pieces. There are 25 pieces for each

Table 1. Light intensity levels (photosynthetically active radiation, PAR) for the four light treatments (µmol m^{-2} s^{-1}).

Source of light	Light level 1 (L1)	Light level 2 (L2)	Light level 3 (L3)	Light level 4 (L4)
Light from LED[1] (n = 5)	726 ± 2	260 ± 3	68 ± 2	20 ± 0
Natural sunlight in the glasshouse[2] (n = 24: 3 times/day ×8 days)	318 ± 33	318 ± 33	318 ± 33	318 v ±33
Total light intensity[(1+2)]	1044	578	386	338

[1]Each value is a mean ± s.e of 5 readings measured in total darkness at representative locations for each light treatment.
[2]Each value is a mean ± s.e. of 24 readings measured at 10.00–10.30 am, 12.30–1.00 pm and 3.30–4.00 pm at representative locations in the glasshouse that did not receive any light from LED light.

experimental unit that were placed into a 150 mm ×200 mm, 20-micron microperforated polypropylene storage bag (Polybags Ltd, Greenford, United Kingdom).

The samples were stored in a randomised order in a walk-in refrigerator at 5.0 °C under dark conditions for eight days before analysis. Eight days of storage was chosen to maximise pinking before microbial decay, with a few days being added compared to typical retail best-before date to adjust for time lags and breaks in the supply chain in a commercial setting.

Two set of leaf samples were prepared; one for day 0 timepoint and another set for day 8. For day 0, the pinking index of the midribs was assessed immediately after processing, and then the samples were freeze-dried and kept at −80 °C until required for further analysis. For the second set of samples, the midribs were stored at 5°C before being scored for pinking indices, freeze-dried and kept at −80 °C until further use for metabolite analysis.

Pinking index

Discolouration of the midrib determination in the term of the pink index was carried out on day 0 and day 8 of storage. This was assessed using the objective discolouration on a 5-point scale with early symptoms of discolouration being 0 (clean), 1 (slightly coloured), 2 (slightly pink), 3 (mid pink) and 4 (intense pink) (modified from Hilton et al., 2009) were scored. On the day of the pinking assessment, the number of midribs with signs of pinking discolouration (pinking incidence) for the 25 quadrat sections drawn on an acrylic sheet placed on top of the storage bags of midribs was counted and the score of the most intense colour of each quadrat sections was recorded. The pinking index was then calculated by multiplying pinking incidence (number of quadrats with pinking), and pinking intensity (pinking score). The pinking index would range from 0 to 4.

Determination of phenolic acids

Phenolic acid determination in the midrib extracts was conducted using High-Performance Liquid Chromatography with Diode-Array Detector (HPLC-DAD) using Agilent 1100 with DAD system (Agilent, Santa Clara, CA, USA). The freeze-dried midrib samples were ground into a fine powder using Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ, USA), sieved and stored in a

−80 °C freezer before extraction. The extraction of the samples was done as follows: 40 mg of midrib powder was added with 1.5 mL of 90% aqueous methanol (v/v) before vortexing for 10 sec, sonicating for 1 min, followed by centrifugation at 4 °C at 13,000 ×g for 20 min. The supernatant was filtered with a 0.2 um filter into Eppendorf tubes. 400 µL of extract was added into HPLC vials and stored at −80 °C. At the time of analysis, 20 µL of isovanillic acid was added to the HPLC vials as an internal standard. The mobile phase was acetonitrile + 0.1% formic acid (Solvent A) and HPLC-grade water + 0.1% formic acid (Solvent B) with a gradient of A:B; 5:95 at 0–5 min, 50:50 at 40 min, 100:0 at 55–55.9 min, and 5:95 at 60 min at 26 °C column temperature at 1 mL min^{-1}. The stationary phases were a ZORBAX Eclipse Plus C18 column (2.1 ×150 mm, 3.5 µm) (Crawford Scientific, Strathaven, UK). DAD analysis wavelength was set at 280 nm for cinnamic acid and 320 nm for the rest of the phenolic acids. External standards used were methanol-diluted cinnamic acid, coumaric acid, caffeic acid and chlorogenic acid.

Determination of soluble sugars

Soluble sugars were determined using Ion Chromatography Mass Spectrometry (IC-MS) with Dionex ICS 6000 (Thermo Fisher Scientific, Sunnyvale, CA, USA). 1.5 mL of 0.01 M HCl was added to 40 mg of lettuce powder before vortexing for 10 sec, sonicating for 1 min, before centrifugation at 4 °C at 13,000 ×g for 20 min. The supernatant was filtered with 0.2 µm filter into Eppendorf tubes. 500 µL of supernatant was added into amber HPLC vials and stored at −80 °C. Before analysis, 25 µL of 20 mM rhamnose was added to the HPLC vials as an internal standard, diluted to 2.25 mM. The IC-MS analysis was done using mobile phases 500 mM NaOH (Solvent A), 16 mM NaOH (Solvent B and C), and milli-Q distilled water (Solvent D). The eluent gradient of A:B:C:D is; 0:50:50:0 at 0–30 min, 50:0:0:50 at 30–40 min, and 0:50:50:0 at 40–55 min at 20 °C column temperature at 1 mL min^{-1} flow rate. The stationary phase was a Dionex Carbopac^{TM} PA1 column (4 ×250 mm, Analytical, Thermo Fisher Scientific, Sunnyvale, CA, USA). The electrochemical detector (ED) cell was set at palladium-hydrogen (PdH) for reference electrode (CE); eluent type Base; detection at 30 °C at 10 mM concentration. The waveform settings used were the default settings for carbohydrate determination: Gold, PdH RE, Carbon, Quad. External standards used were glucose, galactose, fructose, and sucrose diluted in 0.01 M HCl.

Determination of total ascorbic acid

Total ascorbic acid determination was conducted using High-Performance Liquid Chromatography with a Diode-Array Detector (HPLC-DAD) using Agilent 1100 with a DAD system (Agilent, Santa Clara, CA, USA). 1.0 mL of 3.3% ice-cold metaphosphoric acid (MPA) (w/v) was added to 40 mg of

lettuce powder. The mixtures were then vortexed, sonicated, and centrifuged at 4 °C at 13,000 ×g for 20 min before being stored in amber HPLC vials to prevent oxidation. Before analysis, in 100 µL of supernatant, 50 µL 5 mM dithiothreitol (DTT) in phosphate-buffered saline (PBS) was added to start reducing the reaction and left for 15 min in a dark room. Following that, 50 µL 8.5% (w/w) of aqueous *ortho*-phosphoric acid was added to stop the reduction. About 10 µL of isoascorbic acid was added as an internal standard. HPLC-DAD analysis was done using 3 mM potassium dihydrogen phosphate as the mobile phase with an isocratic eluent gradient at 0–10 min at 25 °C column temperature at 0.5 mL/min. The column used was ZORBAX Eclipse Plus C18 (2.1 ×150 mm, 3.5 µm) (Crawford Scientific, Strathaven, UK). DAD analysis wavelength was set at 248 nm. External standards used were ascorbic acid and dehydroascorbic acid diluted in 3.3% MPA (w/v), both stabilised with 5 mM DTT.

Data analysis

Data collected were subjected to ANOVA analysis using SAS Statistical Package ver. 9.4 (SAS Institute, Cary, NC, USA). The treatment factors were arranged in a randomised complete block design (RCBD) with three replicates. Means comparison among treatments were compared using Tukey's HSD test at $p < 0.05$. To explore the association between independent variables, principal component analysis (PCA) was performed using Minitab (Minitab, LLC). Principal components with eigenvalues of more than 1.0 were included in the report.

Results

Pinking index

The pinking index of the leaf midrib at the beginning of the study (day 0) were all 0 for Icarus and 0 to 0.10 for Keona (Table 2). With these pinking indices, the midribs of both cultivars under all light intensities were regarded as 'clean'.

On day 8, the pinking index of mibribs of Keona was consistently higher than for Icarus at all intensities. The pinking of the midribs in plants grown under the highest light intensity (L1, 1044 µmol m^{-2} s^{-1}) was the highest for Keona and Icarus; the values then decreased with decreasing light intensity. Reduction in pinking of midribs in plant grown in L2 (578 µmol m^{-2} s^{-1}) and L3 (386 µmol m^{-2} s^{-1}) for Keona was not differed significantly. There was also no significant different in the pinking of Icarus in L3 and L4 (338 µmol m^{-2} s^{-1}). There was large difference in the pinking of Keona between those grown in L3 and L4.

The increase in pinking index from day 0 to day 8 for Keona was larger compared to Icarus as clearly depicted in Table 2. At 8 days of storage, the overall pinking index for Keona was 2.37 and the corresponding value for Icarus was 1.21 at a possible index maximum of 4.00, regardless of light intensity. The pinking index across cultivars decreased by 30.0, 58.3 and 76.1% when light intensities were reduced from L1 to L2, L3 and L4 plants.

Phenolic acids

Four types of phenolic acids (cinnamic acid, coumaric acid, caffeic acid and chlorogenic acid) that form along the phenylpropanoid pathway, which are linked to the pinking of lettuce, were determined in the study using HPLC-DAD analysis (Figure 2).

Table 2. Means of pinking index of midribs of two cultivars of Romaine lettuce as affected by light level and storage.

Light intensity (µmol m^{-2} s^{-1})	Cultivar	Days of storage	Pink index
L1 (1044)	Icarus	0	0.00f
		8	2.13c
	Keona	0	0.07f
		8	3.28a
L2 (578)	Icarus	0	0.00f
		8	1.30d
	Keona	0	0.10f
		8	2.68b
L3 (386)	Icarus	0	0.00f
		8	0.65e
	Keona	0	0.03f
		8	2.46b
L4 (338)	Icarus	0	0.00f
		8	0.75e
	Keona	0	0.00f
		8	1.05de

Values followed by similar letter(s) did not differ significantly according to Tukey's HSD at $p < 0.05$.

Cinnamic acid

Keona grown in L1 (1044 µmol m^{-2} s^{-1}) produced leaves with the highest initial concentration of cinnamic acid at day 0 (0.22 µmol g-1 DW) in the midribs and the metabolite concentration decreased with decreasing levels of light intensity (Figure 2a). There was no difference in the initial cinnamic acid concentration between those planted in L2 (578 µmol m^{-2} s^{-1}) and L3 (386 µmol m^{-2} s^{-1}); and between L3 and L4 (338 µmol m^{-2} s^{-1}). The initial concentration of cinnamic acid in Icarus grown in L1 was lower than Keona which was grown under similar light intensity (0.15 µmol g^{-1} DW). As for Keona, the cinnamic acid concentration decreased with decreasing light intensity.

The concentration of cinnamic acid in the midribs of Keona grown in L1 and L2 was not affected by storage. However, the concentration of cinnamic acid in Keona for midribs of plants grown in L3 and L4 increased during storage. For Icarus, the concentration of cinnamic acid grown in L1 remained unchanged during storage but increased for those grown in L2. There was no difference in cinnamic acid concentration for plants grown in L3 during storage before it increased again under L4.

Coumaric acid

Changes in coumaric acid concentration during the period of storage varied between Keona and Icarus cultivars (Figure 2b). In Keona, the concentration of coumaric acid in midribs increased from 1.40 on day 0 to 3.29 µmol g^{-1} DW on day 8, (an increase of 135.0%) compared to those of Icarus which has increased from 1.21 to 2.17 µmol g^{-1} DW (an increase of 96.0%) for the same period across light intensities.

The increase in coumaric acid concentration from day 0 to day 8 was more apparent in leaves produced at higher light intensities (L1, 1044 µmol m^{-2} s^{-1} and L2, 578 µmol m^{-2} s^{-1}) compared to those grown under lower light intensities (L3, 386 µmol m^{-2} s^{-1} and L4, 338 µmol m^{-2} s^{-1}). There was no significant difference between the concentration of coumaric acid in midribs of plants grown in L1 and L2, or between plants grown in L3 and L4. The concentration of the coumaric acid on day 0 for L1, L2, L3 and L4 were 1.59, 1.39, 1.19 and 1.03 µmol g^{-1} DW across cultivars, respectively. The corresponding values for day 8 of storage were 3.32, 3.42, 2.05 and 2.1 µmol g^{-1} DW.

Caffeic acid

Leaf midribs of Keona contained a higher concentration of caffeic acid than Icarus's midribs (Figure 2c). On day 0, the caffeic acid concentrations in Keona and Icarus were almost similar to their averages (4.37 and 4.28 µmol g^{-1} DW, respectively) across all light intensities. The concentration of caffeic acid in the midribs of Keona and Icarus were markedly different at day 8 of storage with the respective values of 6.21 and

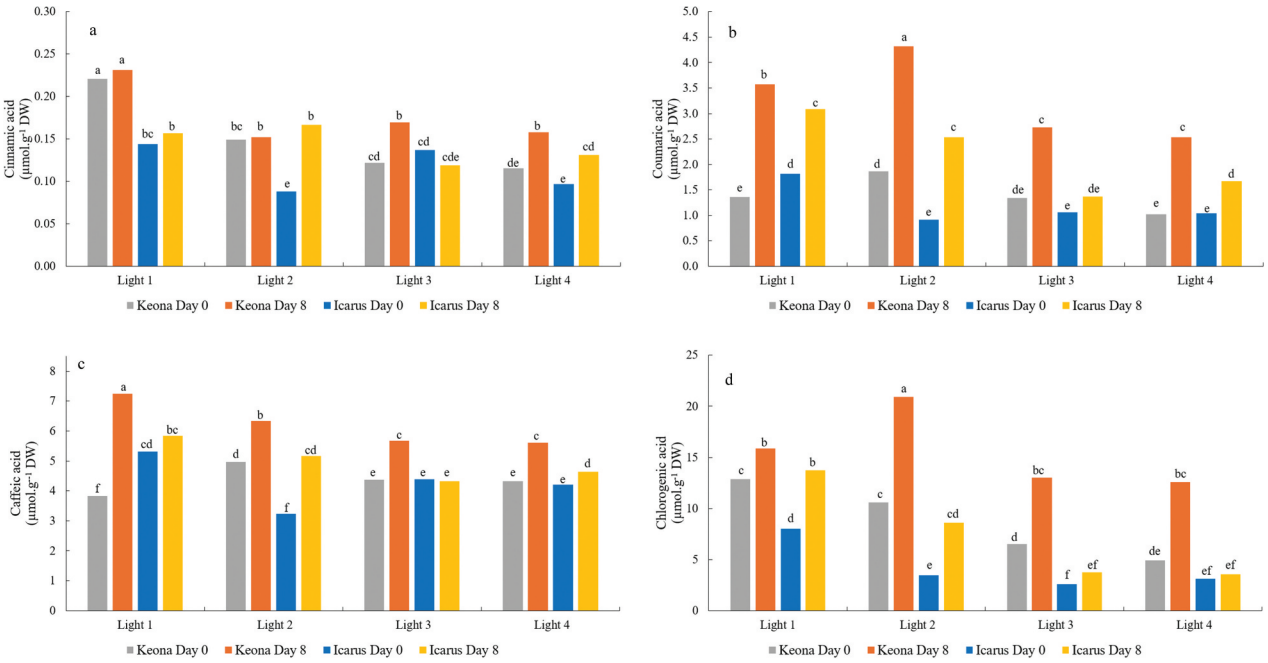

Figure 2. Cinnamic acid (A), coumaric acid (b), caffeic acid (c) and chlorogenic acid (d) of two cultivars of Romaine lettuce at day 0 and day 8 of cold storage (5 °C). Keona day 0 - Keona at day 0 of storage; Keona day 8 - Keona at day 8 of storage; Icarus day 0 - Icarus at day 0 of storage; Icarus day 8 - Icarus at day 0 of storage. Light 1–1044 µmol m^{-2} s^{-1}, light 2–578 µmol m^{-2} s^{-1}, light 3–386 µmol m^{-2} s^{-1} and light 4–338 µmol m^{-2} s^{-1}. Bars with similar letters indicate no significant difference according to Tukey's HSD at $p < 0.05$.

4.99 μmol g^{-1} DW; an increase of 42.1% for Keona and 16.6% for Icarus.

At day 0, the concentration of caffeic acid in leaves of Keona grown in L1 (1044 μmol m^{-2} s^{-1}) was the lowest and Keona grown in L2 (578 μmol m^{-2} s^{-1}) contained the highest concentration of caffeic acid. In contrast, Icarus grown in L1 contained the highest initial concentration of caffeic acid but those grown in L2 contained the lowest concentration of the phenolic acid. The concentrations of caffeic acid of Keona and Icarus grown in L3 (386 μmol m^{-2} s^{-1}) and L4 (338 μmol m^{-2} s^{-1}) were similar at day 0. Cold storage for eight days increased the concentration of caffeic acid in midribs of Keona produced in all light intensities. In contrast, significant increases in caffeic acid in midribs of Icarus during storage occurred in L1, L2 and L4, and in L3.

Chlorogenic acid

The concentration of chlorogenic acid increased over eight days of storage but the increase was dependent on light intensity and cultivars. For L1 (1044 μmol m^{-2} s^{-1}) and L2 (578 μmol m^{-2} s^{-1}), significant increases in chlorogenic acid from day 0 to day 8 occurred in both cultivars. When grown in L3 (386 μmol m^{-2} s^{-1}) and L4 (338 μmol m^{-2} s^{-1}), the increase in chlorogenic acid was only significant for Keona but not for Icarus (Figure 2d). Results obtained also revealed that the concentration of chlorogenic acid was highly affected by light intensity. Regardless of cultivars, chlorogenic acid contents in plants grown in L1 were not significantly different from those grown in L2 while those grown in L3 were similar to the ones grown in L4.

Soluble sugars

To assess the importance of sugars in relations to pinking in two cultivars with contrasting pinking sensitivity grown under varying light intensities four type of soluble sugars; glucose, galactose, fructose and sucrose in midribs were determined (Figure 3).

Glucose

Results obtained clearly show that the concentration of glucose was significantly affected by cultivars and light intensities, and the concentration of glucose was markedly reduced during storage (Figure 3a). Keona contained a higher concentration of glucose than Icarus regardless of light intensity and storage. On day 0, glucose concentration in Keona grown in L1 (1044 μmol m^{-2} s^{-1}) and L2 (578 μmol m^{-2} s^{-1}) were similar, and its concentrations were reduced in L3 (386 μmol m^{-2} s^{-1} and L4 (338 μmol m^{-2} s^{-1}). There was a significant difference in glucose concentration between L3 and L4. For Icarus, like Keona – the concentration of glucose for L1 and L2 plants were similar (no significant difference) on day 0. However, the glucose concentration for L3 and L4 was significantly lower than those contained in the midribs of L1 and L2 plants.

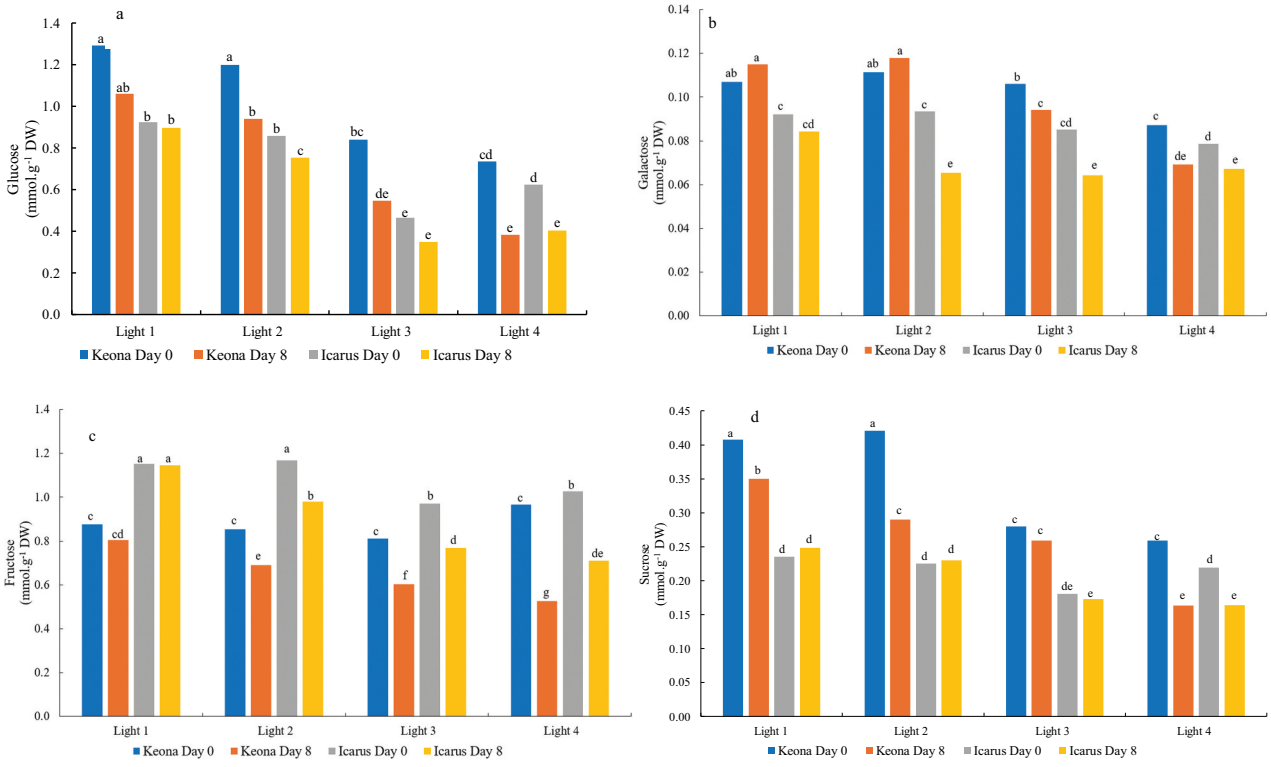

Figure 3. Glucose (a), galactose (b), fructose (c) and sucrose (d) of two cultivars of Romaine lettuce at day 0 and day 8 of cold storage. Please refer to Figure 2 for the details.

The concentrations of glucose were reduced during the eight days of storage. However, the reduction in glucose in the midribs of L1 plants was not significantly different from those of L2 plants for Keona. In contrast, the concentration of glucose in midribs of L2 plants was reduced significantly for Icarus. Glucose concentrations in midribs of plants grown in L3 and L4 were consistently reduced during storage for both cultivars.

Galactose

The initial concentrations (day 0) of galactose in midribs of Keona grown in L1 (1044 µmol m^{-2} s^{-1}), L2 (578 µmol m^{-2} s^{-1}) and L3 (386 µmol m^{-2} s^{-1}) were similar and this was significantly different from leaves of plants grown in L4 (338 µmol m^{-2} s^{-1}). On the same day of analysis (day 0), the concentration of galactose in Icarus was consistently lower than those recorded for Keona.

The concentration of galactose for L1 and L2 plants did not change during storage for Keona, and significant reductions in the sugar for the cultivars were only detected for L3 and L4.

Conversely, the changes in galactose concentration in Icarus did not occur in the midribs of plants grown in L1, but reductions in galactose concentration occurred for all plants grown in other light intensities (L2, L3 and L4).

Fructose

The concentrations of fructose in Icarus were higher than in Keona at day 0. Fructose concentrations at day 0 in L1 (1044 µmol m^{-2} s^{-1}) and L2 (578 µmol m^{-2} s^{-1}) were not significantly different but their concentrations were markedly different from those recorded for L3 (386 µmol m^{-2} s^{-1}) and L4 (338 µmol m^{-2} s^{-1}). In contrast, the concentrations of glucose for Keona in L1, L2, L3, and L4 were similar (Figure 3c).

Eight days of storage did not affect the concentrations of fructose in the midribs of L1 plants for both Keona and Icarus. Cold storage however reduced the concentrations of fructose significantly when the plants were grown at L2, L3, and L4 for the two cultivars.

Sucrose

Analysis of sucrose for day 0 shows that midribs of Keona grown in L1 (1044 µmol m^{-2} s^{-1}) and L2 (578 µmol m^{-2} s^{-1}) produced the highest concentrations of sucrose (0.407 and 0.420 mmol g^{-1} DW, respectively for L1 and L2). Its concentrations were reduced in midribs of plants grown in L3 (386 µmol m^{-2} s^{-1}, 0.280 mmol g^{-1} DW) and L4 (338 µmol m^{-2} s^{-1}, 0.259 mmol g^{-1} DW) (Figure 3d). On the same date, contrasting results were recorded for Icarus whereby the concentrations of sucrose for the cultivar grown under all levels of light intensity produced insignificant concentration of sucrose, which ranged from 0.180–0.235 mmol g-1 DW.

Upon the eighth of storage, a significant reduction in sucrose concentrations for Keona occurred in the leaves of plants grown in L1, L2, and L4. Reduction in sucrose concentrations in Keona for L3 during storage was also observed but its concentration on day 8 did not differ significantly from those on day 0. There was no significant change in the concentrations of sucrose observed in Icarus during the eight days of storage.

Ascorbic acid

The initial concentration (on day 0) of total ascorbic acid (TAsA) in midribs of Icarus grown in L1 (1044 µmol m^{-2} s^{-1}), L2 (578 µmol m^{-2} s^{-1}), and L3 (386 µmol m^{-2} s^{-1}) were higher than those recorded for Keona (Figure 4). The concentration of TAsA for Keona in the midribs of plants grown in L1, L2, and L3 were similar, and all of them were significantly higher than the TAsA concentration in plants grown in L4 (338 µmol m^{-2} s^{-1}). TAsA in midribs of Keona produced in L1 was not affected during the cold storage. However, the concentrations of TAsA in midribs of plants of other treatment combinations (Keona in L2, L3 and L4, and Icarus in L1, L2, L3 and L4) were reduced over the eight days of storage.

Principal component analysis

Principal component analysis (PCA) was performed in order to deduce and identify clusters of the response variables across light intensity and storage period for both cultivars. Two separate analyses were carried out for phenolic acids and sugars. In each case, ascorbic acid and pinking index were incorporated to explore the associations between variation in ascorbic acid and pinking index with phenolic acids and sugars, respectively. Correlations with loadings values more than 0.40 were considered as having significant correlations with their respective principal component (PC) and among traits within each PC.

Phenolic acids

Results of PCA for phenolic acids are summarised as score plot, loading plot (Figure 5) and eigenvectors (Table 3). Application of PCA, as shown by distribution of elements in score plot clearly indicated that individuals (leaf midribs) can be grouped according to the treatments. As shown in Figure 5, total variance accounted for Keona and Icarus were 78.2 and 80.2%, respectively for both principal components.

For Keona, most of the individuals for samples that have been subjected to high light intensities (L1, 1044 µmol m^{-2} s^{-1}; L2, 578 µmol m^{-2} s^{-1}) are mainly situated in 1st and 2nd quadrants with high scores concerning PC1. Similarly, elements for cut lettuce leaves after 8 days of cold storage are also mainly located in the 1st and 2nd quadrants (red-circled,

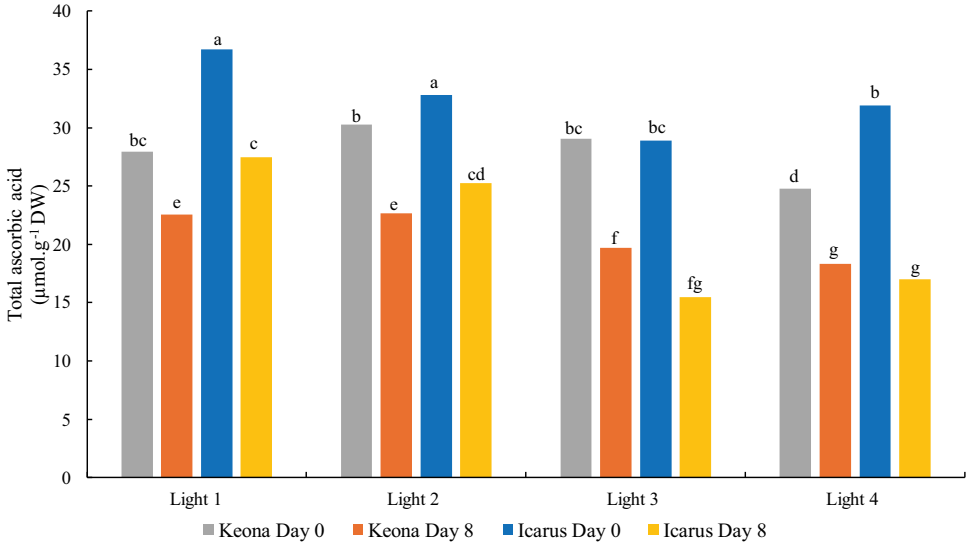

Figure 4. Total ascorbic acid of two cultivars of Romaine lettuce at day 0 and day 8 of cold storage (5 °C). Please refer to Figure 2 for details.

Figure 5a) with positive values with respect to PC1, while those with those under 0-day of cold storage are located in the 3rd and 4th quadrants (blue-circled, Figure 5a).

For Icarus, clear separation between individuals based on the treatments given was seen in those grown in the highest (L1) and the second highest light (L2) intensities and after eight days of storage with respect to PC1 (red-circled, Figure 5b). Data points belonging to lettuce grown under low light intensities mainly existed in 3rd and 4th quadrants with negative values of PC1 (blue-circled, Figure 5b).

The main purpose of PCA was to determine the associations or correlations between traits. Results shown in Figure 5 depict the correlation between phenolic acids, TAsA and pinking index for Keona and Icarus. For Keona, coumaric acid, caffeic acid, chlorogenic acid and pinking index were strongly correlated with PC1. The results suggest that all these traits will change together in a similar direction when a change in profile occurs in one of them. In addition, cinnamic acid and TAsA were found to correlate positively with PC2.

The results for Icarus were similar to Keona, whereby coumaric acid, caffeic acid, chlorogenic acid and pinking index were positively correlated with PC1. Unlike Keona, the correlation between cinnamic acid with other phenolic acids was stronger in Icarus. Compared to Keona, the negative relationship between total ascorbic acid and pinking was weaker in Icarus. Only TAsA was strongly correlated with PC2.

Soluble sugars

Results of PCA for sugars shown as score plot and loading plot, and eigenvectors are shown in Figure 6 and Table 4, respectively. As for phenolic acids, elements in score plots clearly indicated that individuals (leaf midribs) can be grouped according to the treatments. For Keona, individuals grew under the highest light intensity located along the right-hand side (with positive scores) of PC1, while those grown under low light intensity mainly existed on the left (with negative scores with respective to PC1; blue-circled, Figure 6a). Most of the individuals who had undergone 8 days of storage had positive values along the PC2 (red-circled, Figure 6a), while individuals who had undergone 0 days of storage had negative values in PC2.

The distribution of individuals for Icarus was somewhat different from those for Keona. While individuals for leaves produced under the highest light intensity are mainly situated in 1st quadrant

Table 3. Eigenvectors (correlation coefficients) for phenolic acids, total ascorbic acid, and pinking index with respect to the two principal components (PCs).

	Keona		Icarus	
Traits	PC1	PC2	PC1	PC2
Cinnamic acid	0.317	0.605	0.383	−0.129
Coumaric acid	0.471	0.016	0.497	−0.072
Caffeic acid	0.443	−0.122	0.445	0.244
Chlorogenic acid	0.464	0.215	0.468	0.206
Total ascorbic acid	−0.255	0.746	−0.080	0.930
Pinking index	0.448	−0.124	0.427	−0.106

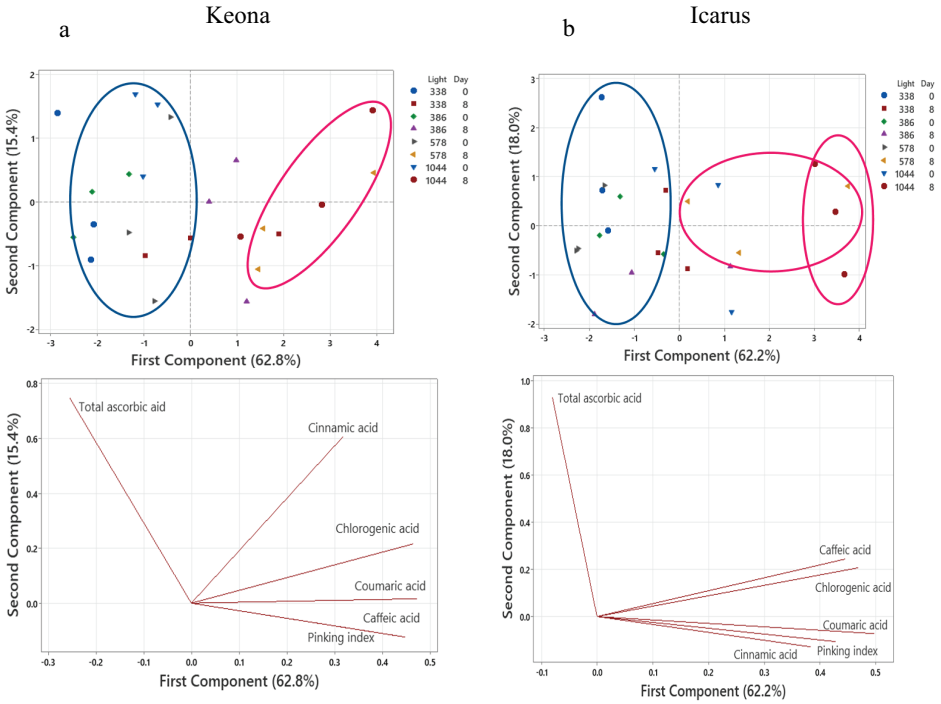

Figure 5. Score plot (top) and loading plot (bottom) of principal component analysis (PCA) of phenolic acids, total ascorbic acid and pinking index of two cultivars of Romaine lettuce grown under four light levels and stored at two period of storage. Light level: L1–1044 µmol m^{-2} s^{-1}, L2–578 µmol m^{-2}·s^{-1}, L3–386 µmol m^{-2} s^{-1}, L4–338 µmol m^{-2} s^{-1}; period of storage: d0–0 day, d8–8 days.

in Keona, the individuals for Icarus under the similar light intensity are located in the 2nd quadrant with positive score on PC1 and negative score on PC2 (red-circled, Figure 6b). The grouping with respect to PC2 between storage periods were apparent for individuals produced under different light intensity levels. For instance, individuals of L2-day 0 (green-circled, Figure 6b) were distributed in 1st quadrant, while those of L2-day 8 were distributed in 3rd quadrant (brown-circled, Figure 6b). Similar to phenolic acids, such variations in the position of individuals recorded in this study suggest that sugar profiles were markedly affected by the treatments given as described above.

The total variance accounted for PC1 and PC2 for Keona and Icarus were 67.9 and 69.2%, respectively (Figure 6). In Keona, the PC1, which contributed to 41.2% to the variation was positively correlated with glucose, galactose, and sucrose. The PC2 was strongly positively correlated with pinking index and negatively correlated with fructose and TAsA. The results suggest that the product with high fructose and/or ascorbic acid could have a lower pinking index.

The results for Icarus were similar with Keona in relations to PC1 whereby glucose, galactose and sucrose were correlated with PC1. However, unlike Keona, TAsA did not correlate with PC2, albeit its negative correlation with fructose, suggesting that total ascorbic acid did not influence the pinking development in Icarus.

Discussion

The ability to control light intensity is important in regulating horticultural production in a protected environment, especially in high-latitude regions. Low intensities of light reduce production capability and quality due to low photosynthetic capacity while excessively high light could cause injury to the lettuce plant (Fu et al., 2012; Min et al., 2021; Riga & Benedicto, 2017). When lettuce is exposed to high

Table 4. Eigenvectors (correlation coefficients) for sugar, total ascorbic acid, and pinking index with respect to the two principal components (PCs).

	Keona		Icarus	
Traits	PC1	PC2	PC1	PC2
Glucose	0.608	−0.080	0.597	−0.064
Galactose	0.537	−0.011	0.477	−0.291
Fructose	0.150	−0.484	0.285	0.587
Sucrose	0.558	0.204	0.562	−0.113
Total ascorbic acid	0.052	−0.597	−0.060	0.238
Pinking index	0.074	0.601	−0.124	−0.705

light stress (e.g. more than 800 µmol m^{-2} s^{-1}, Fu et al., 2012), the plants will be subjected to oxidative damage through exposure to reactive oxygen species. Under such situation, the plants initiate various defence mechanisms, including producing more phenolic compounds. In our context, high production of phenolic compounds has been shown by the increase of four phenolic acids measured, i.e. cinnamic acid, coumaric acid, caffeic acid and chlorogenic acid. These four phenolic acids are synthesised along the phenylpropanoid pathway that directly link to the formation of pink discoloration of lettuce (García et al., 2019; Hunter et al., 2017; Saltveit, 2018).

As expected, the pinking of Keona occurred at a higher frequency and intensity, thus giving a significantly a higher pinking index compared to Icarus. Significant differences in pinking between cultivars occurred in L1, L2, and L3 light levels suggesting that the pinking development in lettuce can be effectively regulated by light intensity, and the use of slow and/or low pinking lettuce cultivars such as Icarus offers tangible benefits. At the lowest light intensity used in this study (L4) however, the use of cultivars with low pinking in view of pinking reduction would not be as critical. The reduction in pinking of lettuce produced in low light intensity may be preferred by many actors in the supply chain but the produce that has lowered pinking of midribs after harvest and in fresh-cut produce may come with lower quality attributes such as low sugars and phenolic compounds, and poor texture and taste with short shelf life (Bell et al., 2020; Min et al., 2021; Neugart et al., 2018).

All of the phenolic acids had positive correlations with pinking index as shown by loading plots of PCA (Figure 5) which indicates that increasing in the composition of these compounds would increase the pinking incidence. However, none of phenolic compounds measured are pink in colour and the pinkish colour in lettuce has been reported due to the presence of caffeic acid *o*-quinone (Payne et al., 2006; Saltveit, 2018). Positive correlations between pinking index and phenolic acids observed in this study (Table 3) suggest that caffeic acid *o*-quinone that contributes to the pink discoloration in lettuce must be derived from the phenolic substrates. Although quantification of caffeic acid *o*-quinone seems to be critical for this study, but due to volatility of the compound, its quantification is impractical.

The association between phenolic acids and pinking could vary between cultivars. Cultivars with high pinking tendency could have a higher inclination to synthesise more substrates for PPO while those with low pinking could divert more metabolites for other functions such as for lignification (García et al., 2019; Hunter et al., 2022). In our case, the evidence of higher activity of PPO in high pinking cultivar (Keona), especially when the plants were exposed to high light intensity, could be translated into a weak and even negative correlation between cinnamic acid with other phenolic acids compared to a stronger and positive correlation

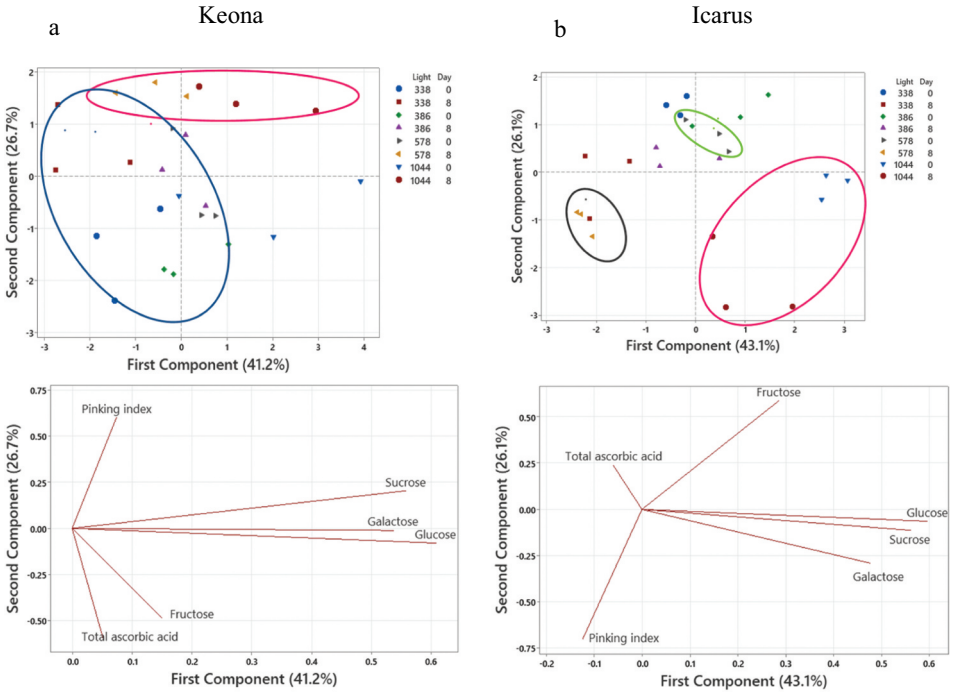

Figure 6. Score plot (top) and loading plot (bottom) of principal component analysis (PCA) of sugars, total ascorbic acid and pinking index of two cultivars of Romaine lettuce grown under four light levels and stored at two period of storage. Please refer to Figure 5 for details.

among phenolic acids measured for Icarus (Figure 5). Weaker correlations between cinnamic acid with other phenolic acids might imply that a higher proportion of cinnamic acid could have been converted to compounds that contributed to pinking development such as caffeic acid-o-quinone. Although PPO does not directly convert cinnamic acid to coumaric acid or to caffeic acid, the continued conversion of caffeic acid to caffeic acid *o*-quinone accelerates the depletion of cinnamic acid as a substrate.

Beside phenolic acids, ascorbic acid was postulated to have played an important role in regulating pink incidence in lettuce according to studies on lettuce pinking (Saltveit, 2018). In our study, TAsA was shown to have a negative correlation with pinking index (Figures 5 and 6) indicative of conversion of *o*-quinone back to colourless *o*-diphenols by through antioxidative activity. Due to this property, AsA has become one of the substances used in controlling oxidative brown discolouration of fruits and vegetables (Moon et al., 2020; Nogales-Delgado, 2021), by competing with PPO, the enzyme that responsible for the oxidation of phenolics, for the substrates (Degl'innocenti et al., 2005; Landi et al., 2013; Wen et al., 2021). Results of negative association between AsA and pinking recorded this study is in-line with earlier reports on the relationship between AsA and browning discolouration (Degl'innocenti et al., 2005; Yu et al., 2023).

Bioynthesis of AsA is a light dependent process (Ntagkas et al., 2019; Riga et al., 2019). Excess light during crop production period may trigger unbalanced redox in plant cells (Y. H. Zhou et al., 2009). To react to such situation, AsA will be rapidly produced to scavenge the reactive oxygen species (W. L. Zhou et al., 2012; Y. H. Zhou et al., 2009), therefore, plants grown under high light such as those under L1 (1044 µmol m^{-2} s^{-1}) and L2 (578 µmol m^{-2} s^{-1}) are expected to contain high concentration of TAsA (Figure 4).

Compared to Icarus, Keona – a cultivar with a higher susceptibility to pinking – contained less TAsA. When subjected to similar growing and storage conditions, plants of this cultivar could theoretically have used a large portion of its AsA to revert back the caffeic acid *o*-quinone to caffeic acid. However, due to a smaller pool of TAsA in Keona and higher pinking incidence (due to high activity of PPO and high availability of pre-cursors especially cinnamic acid and coumaric acid), the amount of TAsA would not be sufficient to reduce the pinking, thus making the pinking of Keona appeared to be higher than that of Icarus. Different patterns of response in production of secondary metabolites including AsA amongst different cultivars of lettuce is a common phenomenon (Gomez & Jimenez, 2020; Woltering & Witkowska, 2016).

Plants grown under high light intensities would produce more photosynthate compared to plants grown in lower intensities, and this is reflected by higher soluble sugar contents in plants. However, increasing light to the highest intensity used in the study (L1, 1044 µmol $m^{-2}s^{-1}$) did not produce any beneficial effect on production of sugar as shown by negligible significant differences in all type of sugars between plants grown in L1 and L2 (578 µmol $m^{-2}s^{-1}$). Such result may be attributed to the light compensation point for CO_2 assimilation during photosynthesis in lettuce which is around L2 light intensity. Fu et al. (2012) found that biomass production of lettuce grown under light level of 600 µmol $m^{-2}s^{-1}$ was higher than the plants grown under 800 µmol m^{-2} s^{-1} and proposed that the light compensation point for lettuce is about 600 µmol m^{-2} s^{-1}. They also concluded that light level of 800 µmol $m^{-2}s^{-1}$ had caused stress to the plants as shown by the increase in the activity of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids produced during the peroxidation of membrane lipids. However, Fu et al. (2012) also reported that the overall concentration of sugar in highlight intensity-stressed plants (grown at 800 µmol m^{-2} s^{-1}) was the highest, as similarly observed in this study. High soluble sugars would affect the flavour of the produce, and this may improve the consumer acceptance of the product, however, high pinking index is likely to deter consumer preference. Therefore, a fine balance between the increase in soluble sugar (and TAsA) and pinking discolouration in lettuce grown under high light intensities must be determined. Given that pink discolouration has not been shown to have any negative impacts on product safety or eating qualities such as taste or texture, a more sustainable approach to management of this disorder may be needed to address the marketing strategies of pink lettuce.

It is interesting to note that the concentration of galactose and fructose did not change significantly during the eight days of storage for plants produced at high light intensities. The results could be associated with the dynamic of sugar pathway whereby glucose, galactose and sucrose are closely linked as shown in PCA loading plots (Figure 6). For example, in plants, galactose is derived from raffinose, and its production is regulated by stress signals. Raffinose itself is derived from sucrose (Nishizawa et al., 2008; S. Yan et al., 2022). When plant received signals from environmental stimuli, such as high light intensity stress as in our case, more raffinose would be generated at the expense of sucrose, and when needed, raffinose will be converted to galactose. Similarly, sucrose will also be converted to glucose for physiological uses such as in increased respiration rate when the plants are stressed,

and under reduced photosynthetic capability (Vanlerberghe et al., 2020). It is also interesting to note that in plants, galactose have a direct linkage with AsA as the AsA is mainly synthesised from D-mannose/L-galactose through the Smirnoff – Wheeler pathway (Ntagkas et al., 2018; Smirnoff, 2018). AsA is also generated *via* glucose and myo-inositol pathways (Ntagkas et al., 2018).

The beneficial effects of high light intensity in increasing carbohydrate and other health promoting compounds such as AsA, phenolics and flavonoids can also be obtained through a shorter exposure of crops to supplementary light for a short period of time. Y. H. Zhou et al. (2009) reported that the compositions of soluble sugars and starch increased by 1.5-fold when lettuce were supplemented with high intensity of HPS light (1000–1200 μmol m^{-2} s^{-1}). Samuolienë et al. (2011) revealed that application of supplemental red light (from LED) at 300 μmol m^{-2} s^{-1} increased TAsA in lettuce. High light intensity was also beneficial in extending the stability of AsA and carbohydrate contents during cold storage, thus prolonging the shelf life of the produce.

In this study, PCA results revealed that TAsA was not correlated with soluble sugars and this was true for Keona and Icarus (Figure 6). This result was in contrast to some earlier research findings that explore the regulation of TAsA by light intensity (Min et al., 2021; Pérez-López et al., 2015; Schmitz et al., 2014). Exposure to high light intensity is known to increase the activity of many enzymes and trigger expression of genes that are involved in AsA biosynthesis and recycling pathways in plants (Bartoli et al., 2006; Dowdle et al., 2007; Yabuta et al., 2007) which could be mediated by photosynthesis and respiration (Bartoli et al., 2006; Ntagkas et al., 2018). The results obtained however is in-line with those reported by Massot et al. (2010) and Ntagkas et al. (2019) for tomatoes, suggesting that the association between AsA and soluble sugars or carbohydrates in other studies could have been dependent on crop species or varieties of a crop species.

In this study, information on biomass accumulation for each growing condition was not collected. It is well established that the total biomass of lettuce grown under low light intensity is reduced due to low photosynthetic capabilities (Boros et al., 2023; Miao et al., 2023; Mohamed et al., 2021). Miao et al. (2023) reported that fresh weight of shoot and root, soluble sugar, soluble protein, and ascorbic acid contents in the crop were elevated with increasing light intensity and *vice-versa*. In our study, higher photosynthetic capability of lettuce plants grown under high light intensities was reflected by a higher concentration of soluble carbohydrates in lettuce plant tissues. Besides carbohydrates, high light intensity also enhanced the production of secondary metabolites measured (i.e. TAsA and phenolic acids). Increases in these metabolites coupled with heavier biomass would mean higher economic yield, which could be advantageous, as well as containing a higher concentration of health-promoting phytochemicals that may function as antioxidants.

Conclusion

Results of this study clearly suggest that cold storage increased the concentration of phenolic acids but concomitantly reduced TAsA and sugar contents in the midribs of lettuce tested. Increased phenolic compounds in vegetables after storage would normally be associated with increased antioxidant activity, therefore elevating the health-promoting capability of the produce. However, this occurred at the expense of ascorbic acid and sugars and was associated with increased pinking incidence. Therefore, a fine balance between increased phenolic contents, reduced AsA and sugars, and acceptable pinking level must be sought out, for example by using the right cultivar, growing under an appropriate environment and storing for a suitable period. Under low light, the choice of cultivars in relation to pinking would not be critical, but biomass accumulation is likely to be compromised. Using slow-pinking cultivars such as Icarus could be a good strategy to produce lettuce with good shelf-life properties, especially when the crops are to be grown under high or medium light levels (PAR level between 400–800 μmol m^{-2} s^{-1}).

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Disclosure statement

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