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Identification of romaine lettuce (*Lactuca sativa* var. *longifolia*) Cultivars with reduced browning discoloration for fresh-cut processing

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ABSTRACT

Discoloration (browning) represents a major challenge that limits the quality and shelf life of fresh-cut lettuce. In this study, we aimed to find romaine lettuce (*Lactuca sativa* var. *longifolia*) accessions with low browning potential. Midribs of 14 accessions (11 cultivars, two breeding lines, and a single plant introduction) were shredded and packaged in perforated bags for five days. Images of processed samples were captured daily and analyzed with computer vision technology to quantify browning intensity via L*a*b* color values and browning index (BI). Enzymatic activity[phenylalanine lyase (PAL), peroxidase (POD), and polyphenol oxidase (PPO)] and total phenolic content (TPC) were measured daily. After five days in storage, the accessions in the Tall Guzmaine and Parris Island Cos pedigree groups exhibited the greatest and least browning, respectively. In addition, while the PAL, POD, and TPC increased substantially over time, the PPO of twelve accessions fluctuated with only minor increases. For all accessions, the temporal increase of PAL, POD, and TPC showed significant, positive correlation to browning progression. Comparing between accessions, those that had greater amounts of accumulated PAL and smaller amounts of POD tended to have a greater amount of browning after five days of storage, despite relatively low correlation coefficients. However, the accumulation of TPC and PPO was not correlated to browning severity after five days of storage. This systematic study provides lettuce growers and breeders with guidance for selecting accessions with limited browning, and it supplies researchers in plant physiology and genetics with more information on the roles of enzymes in the lettuce browning process.

1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the most valuable fresh vegetables and is in the top ten most valuable crops in the US, with an annual farm-gate value of over $2.3 billion (LaVigne, 2017). Lettuce is also the most popular, commercially produced leafy vegetable worldwide (Simko et al., 2014). Fresh-cut lettuce is the primary ingredient of the increasingly popular, packaged, ready-to-eat salads. Discoloration (browning) represents a major challenge that limits the quality and shelf life of packaged lettuce. The lack of effective browning control has resulted in processors relying on modified atmosphere packaging (MAP) to achieve low oxygen atmospheric conditions and maintain the shelf life. However, this induces other problems, especially the development of off-odors and physiological disorders that often occur at extremely low O_2 and high CO_2 levels during the end of the shelf life (Kim et al., 2005a, b; Luo, 2007). Furthermore, low oxygen conditions coupled with high temperature abuse promote population growth and increased virulence factor gene transcription of the human pathogenic bacteria, *Escherichia coli* O157:H7 (Chua et al., 2008; Sharma et al., 2011). It may be possible to reduce food safety risks and increase quality in fresh-cut salads by using lettuce cultivars that tend to have less browning without MAP treatments.

In the last few years, lettuce breeding research has been transitioning from data-poor to data-rich. Vast, unprecedented amounts of genetic, phenotypic, physiological, and economic information have started becoming available from a variety of sources. For instance, genomic approaches were applied to identify the chromosomal location of a *qSL4* locus that plays a critical role in the deterioration rate of fresh-cut lettuce (Simko et al., 2018). Metabolomic analysis of thirty lettuce cultivars has provided valuable datasets of phenolic, lipid, and terpene compounds relevant to the browning process (García et al., 2018). García et al. (2018) found that caffeoylquinic acid and 3-

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hydroxy-tetradecadienoic acid could be used as early biomarkers for greater lettuce browning, while ferulic acid methyl ester and 2-O-p-hydroxyphenyl-6-O-galloyl glucose were identified as indicators for less browning. Hunter et al. (2017) examined the effects of environmental factors and nutrient levels on the physical properties of lettuce leaves, activity of phenylalanine ammonia lyase (PAL), and activity of polyphenol oxidase (PPO). The effect of MAP on the phenolic profile and enzymatic activity of romaine lettuce was studied by Luna et al. (2016), which included the discovery that only specific phenolic compounds are significantly attributable to browning, and that the conversion of PPO from the latent to active form plays a vital role in browning. Browning in twenty-five fresh-cut lettuce cultivars was phenotypically evaluated for differences between storage conditions, which yielded insights into cultivars that are better suited breeding and methods for browning control (Tudela et al., 2016).

Lettuce browning is a typical physiological response to injury or disease. A wound signal is produced upon stress and can be transmitted with the involvement of phospholipase D (PLD) (Choi et al., 2005). PLD generates linolenic acid and phosphatidic acid, which initiates the oxylipin pathway. The products of these reactions, including jasmonic acid (JA), produce signals that trigger the de novo synthesis of PAL, the major enzyme involved in the phenylpropanoid pathway (Dixon et al., 2002). Cinnamic acid is produced with phenylalanine in the presence of PAL, which is further converted to various phenolic compounds. Meanwhile, the wound signal triggers the synthesis of other phenolic compounds, mainly chlorogenic acid (Mai and Glomb, 2013; Tomás-Barberán et al., 1997). The newly synthesized phenolic compounds, together with existing phenolics released from adjacent tissues due to cellular membrane disruption, are oxidized, primarily with the aid of PPO (Landi et al., 2013; Saltveit, 2000). The products of phenolic oxidation are quinones, which undergo polymerization and form colored pigments. The oxidizing activities of PPO can generate peroxide; thus, peroxidase (POD) may also be involved in the process of phenol degradation (Richard-Forget and Gauillard, 1997).

Despite the advancements in research on lettuce browning and enzymes, there has been a discrepancy between the theories and observation. For example, the correlation between enzymatic activity and browning has been controversial. POD and PPO have been considered to contribute to browning (Landi et al., 2013; Richard-Forget and Gauillard, 1997). However, some studies show that the change in PPO activity does not correlate to the progression of browning during lettuce storage (Fukumoto et al., 2002), whereas a positive correlation was found in other studies (Zhan et al., 2012). Similarly, the change in POD activity over time was reported to correlate positively to browning in some cases (Ke and Saltveit, 1989), while the correlation was found to be insignificant in other reports (Cantos et al., 2001; Hyodo et al., 1978). Moreover, since only a limited number of lettuce accessions were evaluated in these conflicting studies, it is unknown to what extent the genetic differences impact the enzymatic activity, browning, and the interactions between them. Additionally, most of these studies are predominantly reliant on visual inspection by trained panelists (Couture et al., 1993; Murata et al., 2004), which is labor-intensive and subject to human error. Alternative methods include absorbance assays (Choi et al., 2005), colorimetric assays (Fukumoto et al., 2002), and digital image analysis (García et al., 2018). Absorbance measurements give results that correlate only moderately to visual inspection. Colorimetric measurements are challenging due to the irregular shape of lettuce samples and the limited number of pieces that can be evaluated per sample. Digital image analysis methods can be difficult due to the three-dimensional, irregular shapes of lettuce pieces that can result in less accurate image segmentation. Furthermore, the definition of "browning" and the impact of human subjectivity in these tests are potential limitations. Lastly, while correlations have been established between changes in enzymatic activities and browning over time, they have rarely been reported in the literature for different cultivars. A comparative assessment of correlations among cultivars is critical to understanding which enzymes account for most of the differences in browning potentials among multiple cultivars and pedigree groups, providing insight into the roles of enzymes from another perspective.

In this study, romaine lettuce from 14 accessions were evaluated for their browning potential along with the underlying enzymatic and chemical mechanisms. The accession selections were based on previous studies that successfully identified cultivars with low (Parris Island Cos) and high (Tall Guzmaine) browning potentials (Hayes and Liu, 2008). A computer vision technique was employed to quantify browning on the midrib tissues. Enzymatic and biochemical parameters including PAL, POD, PPO and TPC were also determined. The availability of pedigree information provided an ideal opportunity to compare biochemical properties and browning in accessions with different genetic backgrounds. The objectives of this study were: (1) to provide lettuce growers and breeders with guidance on cultivar selection, and (2) to advance the understanding of the roles of enzymes in lettuce browning.

2. Materials and methods

2.1. Lettuce sample preparation

Fourteen accessions of romaine lettuce were grown and harvested at the USDA lettuce breeding facilities in Salinas, CA. These accessions included 11 commercial cultivars: Darkland (DL), Green Forest (GF), Green Towers (GT), Hearts Delight (HD), King Henry (KH), Lobjoits (LB), Parris Island Cos (PC), Siskiyou (SK), Sun Valley (SV), Tall Guzmaine (TG), and Triple Threat (TT); two breeding lines: RH11-1506 (RH) and SM13-R2 (SM); and a single plant introduction: PI 491224 (PI). The genetic relationship among those accessions and their relative browning potential determined by analysis of means (ANOM) are illustrated in Fig. 1. Immediately after harvesting, the lettuce was taken to a local distributor and stored in a 5 °C cold room with forced air cooling. The cooled lettuce was transported via commercial refrigerated truck (2–4 °C) to the USDA facilities in Beltsville, MD within three days. The lettuce was then stored at 5 °C for 24 h before sample preparation.

On the day of sample preparation, wrapper leaves and stems were removed manually from each head of lettuce. Lettuce leaf midribs were obtained by carefully excising the green leaves. Representative samples were taken from all locations of each head. The ribs were then cut into 3.2-mm shreds using an industrial vegetable slicer (Nichimo Seven Chefs ECD-302, Tokyo, Japan). Fifty grams of each cut product was placed immediately in a polypropylene perforated bag (17.8 × 18.9 cm^2) and stored at 5 °C for up to five days. Each bag was punctured in three locations with a 20-gauge syringe needle (0.9 mm ID) to allow the ingress of oxygen. The processing and packaging were carried out in triplicate. Disinfection procedure was avoided to preserve enzymes and phenolic compounds. No sign of decay was observed during the period of storage and analysis.

2.2. Quantification of lettuce color and browning severity

Three bags per accession of lettuce were used to quantify the color with a computer vision system. The system included a portable shooting tent with controlled lighting (two light banks with diffusers containing 5600K daylight balanced light emitting diodes), a digital camera (Nikon D 800 with a 60 mm lens, settings: F-20, 1/30 s, ISO 640) fixed perpendicular to the samples, and a computer with image acquisition and analysis software (color correction, segmentation, analysis of pixel colors). For each accession, the contents of the entire bag (50 g lettuce) was spread into a single layer on a matte white background (40.5 x 21.5 cm) and photographed. The imaging was performed quickly, about 2 min per sample, to minimize the effect of temperature abuse in this repeated measures approach to color quantification. The lettuce pieces were then re-packaged, and the content from the same bags were photographed repeatedly after 0, 1, 2, 3, 4, and 5 days of storage at 5 °C.

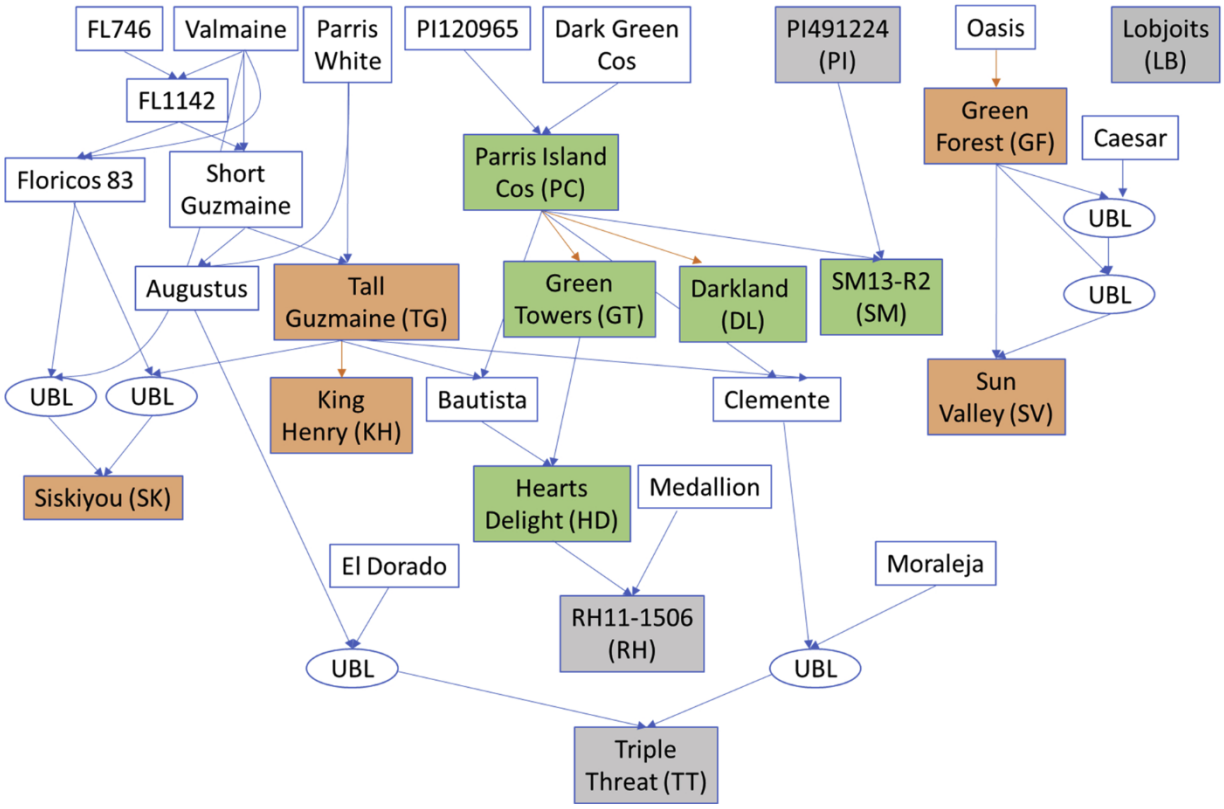

Fig. 1. Complex pedigree relationship among accessions evaluated for browning potential of cut romaine lettuce. Blue arrows indicate the direction leading from parental accessions to their descendant. Blue arrows indicate the direction of selection for accessions that were developed by a single plant selection from another accession. The UBL abbreviation indicate known, but unnamed breeding lines or hybrids in the pedigrees. Accessions tested in this study are colored green (least browning), grey (intermediate browning), and orange (greatest browning). Colors are based on values of Browning Index (BI) at Day 5 (data are shown in Fig. 3C). The accessions highlighted in green and orange represent the ones with the lowest and highest BI, according to analysis of means (ANOM) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Pictures were segmented and analyzed using the smart segmentation tool in Image Pro Premier software (v. 9.3b, Media Cybernetics, Inc., Rockville, MD, USA). A new image segmentation method was developed in this study to classify pixels into six classes [background, regular lettuce ribs (not brown), light brown, medium brown, dark brown, yellow/decayed] based on the variation within and between the defined reference objects for each class. After creating this segmentation method with images from preliminary trials, the method was then applied to each sample picture using the software smart segmentation tool, which compared the pixels in the sample picture to the reference objects and assigned each sample pixel to the class of the closest reference object. The average RGB color values for the pixels in each class from each sample picture were determined using the Image Pro Premier software and converted to L*a*b* (CIELAB 1976) using the Image Processing Toolbox in MATLAB (v. R2017b, MathWorks, Natick, MA, USA). Hue angle (h°) and chroma were calculated from a* and b* values by converting color coordinates from rectangular to polar form.

In addition to these color values, a browning index (BI) was calculated for each lettuce sample as an indicator of visual browning severity in lettuce using

$$BI = \%pixels_{light\ brown} \times 1 + \%pixels_{medium\ brown} \times 2 + \%pixels_{dark\ brown} \times 4 \quad (1)$$

where $\%pixels_{light\ brown}$ is the percent of pixels in the light brown class, $\%pixels_{medium\ brown}$ is the percent of pixels in the medium brown class, and $\%pixels_{dark\ brown}$ is the percent of pixels in the dark brown class. BI increases with increasing browning severity; it incorporates both the total number of pixels with a brown color and the intensity of the brown color, as demonstrated by the proportion of pixels classified into light, medium, or dark brown regions.

2.3. Chemical and enzymatic analysis

Packaged lettuce samples were analyzed for their total phenolic content (TPC), phenylalanine ammonia lyase (PAL) activity, polyphenol oxidase (PPO) activity, and peroxidase (POD) activity on days 0, 1, 2, 3, 4, and 5. The assays were executed following previously reported procedures (Cantos et al., 2001; Lester et al., 2012; Mai and Glomb, 2013). Optimization was performed for throughput improvement, as detailed below. Each of the three replicates was measured twice. Borate buffer with reducing agent, defined as r-BB, (pH 8.5, 50 mmol/L) was prepared from a stock solution (Thermo Fischer Scientific, Waltham, MA, USA) with reducing agent, 2-mercaptoethanol (5 mmol/L). All other chemical reagents were of analytical grade from Sigma Aldrich (St. Louis, MO, USA).

2.3.1. TPC

Samples (10 g) were homogenized in a 50 mL chilled methanol/water mixture (90/10, v/v) for 1 min. The homogenate was filtered with a Whirl-Pak mesh filter bag (23 × 15 cm^2, 200 um pore size, Nasco Inc., Fort Atkinson, WI, USA) and passed through a syringe filter (0.45 µm pore size, VWR International, Radnor, PA, USA). The resultant filtrate (250 u L) was loaded onto a UV-transparent 96-well microplate (Corning Inc, Corning, NY, USA), and the absorbance was measured at 420 nm using a Synergy H1 plate reader (Biotek, Winooski, VT, USA). Afterwards, the sample was mixed with 25 µL fast blue BB (FBBB) solution (1 mg/mL in water), incubated at 30 °C for 1 min, and mixed with 25 µL NaOH solution (50 g/L in water). The resultant mixture was

incubated at 30 °C for 60 min before the second absorbance was measured. A standard curve was established with gallic acid in 80% methanol in water (v/v), and TPC was expressed in gallic acid equivalents (Lester et al., 2012; Mai and Glomb, 2013).

2.3.2. PAL

Samples (20 g) with 2 g polyvinylpolypyrrolidone (PVPP, cross-linked, 110 um particle size) were homogenized in 50 mL chilled r-BB for 1 min. The resulting dispersion was incubated in an ice bath for 30 min, filtered with a mesh bag (200 μm pore size), and further passed through a syringe filter (1 μm pore size, VWR International, Radnor, PA, USA). Except for the day 0 samples, the filtrate was diluted two-fold with chilled r-BB. For each sample, 270 μL of the filtrate was mixed with 30 μL of r-BB containing 100 mmol/L L-phenylalanine on a UV-transparent 96-well microplate. The mixture was incubated at 40 °C for 5 min, and the absorbance was measured at 290 nm. A second measurement was performed after additional incubation at 40 °C for 60 min. As a control, 270 μL of the filtrate was mixed with 30 μL r-BB but without L-phenylalanine and subjected to the same measurement. The overall increase in the absorbance, ΔOD_{290}, was calculated as the increase in OD_{290} of the sample with phenylalanine minus that of the control. One unit (U) of PAL activity was defined as the increase in OD_{290} by 1.00 per hour (Fukumoto et al., 2002).

2.3.3. PPO and POD

Samples (10 g) with 2 g of polyvinylpolypyrrolidone (PVPP) were homogenized in 50 mL of chilled phosphate buffer (PB, pH 6.2, 50 mmol/L) for 1 min. The resulting dispersion was incubated in an ice bath for 30 min, filtered with a mesh bag (200 μm pore size) and further passed through a syringe filter (1 μm pore size). Twenty-five microliters of the filtrate was mixed with 275 μL PB containing either 0.12 mol/L catechol (for PPO) or 4 mL/L guaiacol and 1.33 mL/L H_2O_2 (for POD) on a UV-transparent 96-well microplate. Immediately after the mixing, the absorbance at 410 nm (for PPO) or 470 nm (for POD) was recorded every two seconds for 1 min. The slope of the linear portion of the time curve was calculated. One unit (U) of activity was defined as the increase in OD_{410} (for PPO) or OD_{470} (for POD) by 0.1 within 1 min (Fukumoto et al., 2002).

2.4. Statistics

The data are expressed in mean ± standard error (n = 3). The following statistical analyses were performed using Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA). Analysis of means (ANOM) was performed to identify accessions with high, medium, and low browning potentials. Pearson correlation analysis was used to find the correlation between BI and biochemical indices including PAL, POD, PPO, and TPC. Simple linear analyses were conducted to model the change in BI over time.

3. Results

3.1. Color change during storage

Figs. 2 and 3 depict the time-dependent change in color parameters, with the rates of change summarized in Table 1. A significant decrease in L* values (Fig. 2A) was observed over time ($P < 0.01$, detailed statistical results are shown as Supplementary Table S1), accompanied by a significant increase in a* values (Fig. 2B). These results indicate an increase in the darkness and redness of the lettuce midrib pieces. Most accessions exhibited similar L* and a* values on day 0, a slight change in the first two days, and a rapid change between days 2 and 5. Overall, L* and a* values changed at the greatest rates for the accession TG, followed by KH, SK, and GF (Table 1). In contrast, DL and PC had the smallest changes in L* and a* values during storage.

Unlike a* value, there were greater differences in b* values between some accessions on day 0, reflecting variations in the baseline level of yellowness on the lettuce rib surface (Fig. 2C). During storage, b* values increased significantly and exhibited significant differences among accessions. PI had the largest increase in b* value over time, followed by GF, GT, KH, and SK, with the slope values ranging from 2.0 to 2.5

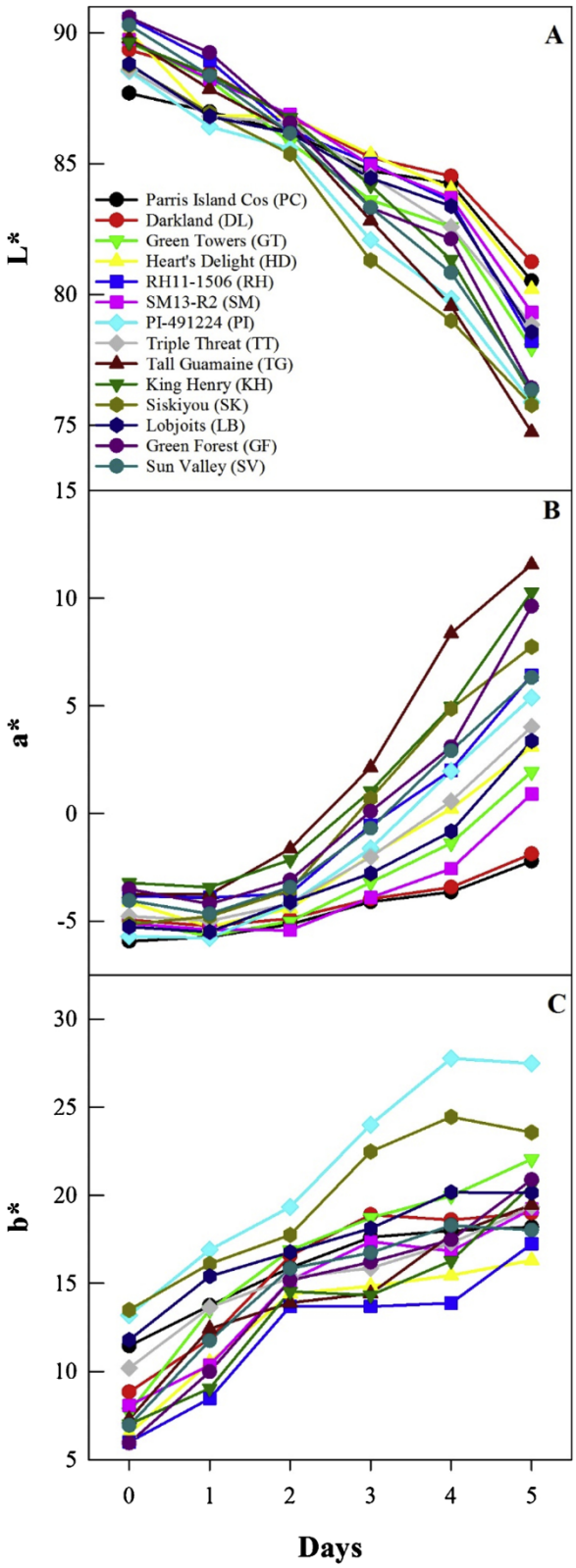

Fig. 2. Change in color parameters L*, a*, and b* of lettuce midrib tissues over time at 5 °C and among lettuce accessions.

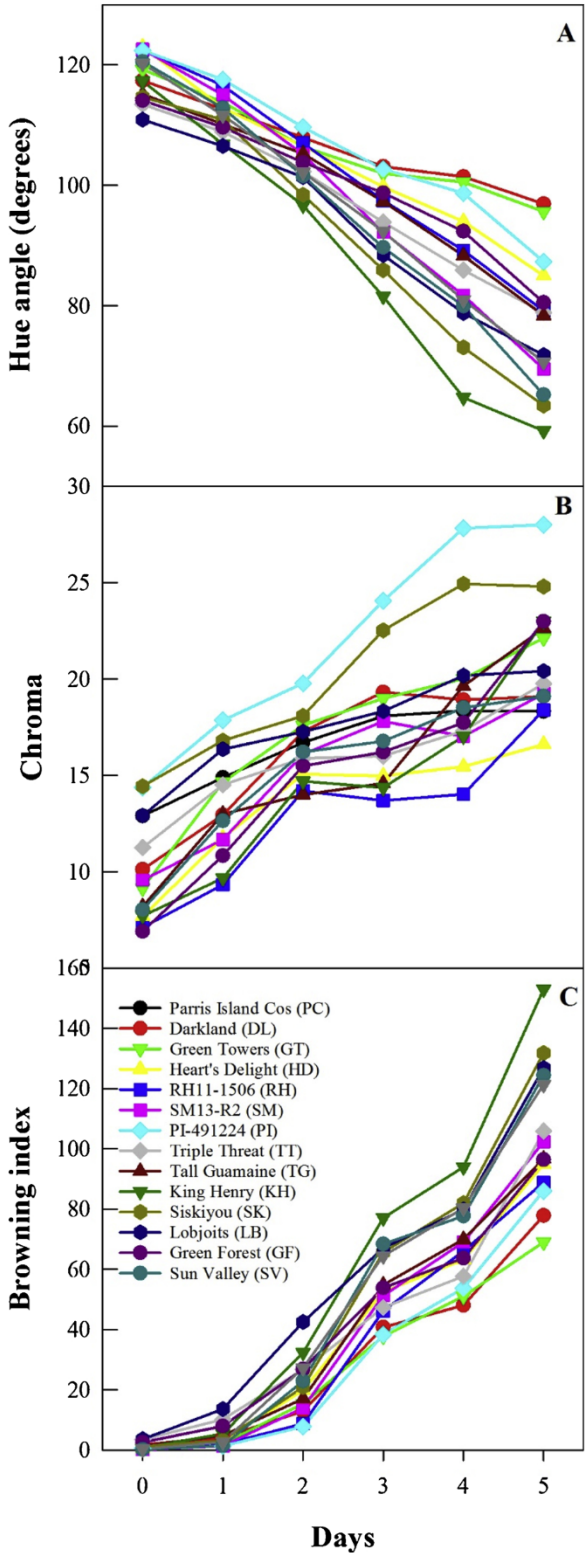

Fig. 3. Change in hue angle, chroma, and browning index over time at 5 °C and among lettuce accessions.

day^{-1} (Table 1). PC had the smallest changes in b*, followed by TT, LB, HD, and DL.

The h° value derived from a* and b* declined during storage at significantly different rates among accessions (Fig. 3A, Table 1). The greatest rate of decline was observed for TG, followed by GF, KH, and RH, with h° slopes ranging from -12.7 to -11.4 degrees day^{-1}. In contrast, the h° values for PC and DL decreased at a slower rate of -3.8 and -4.2 degrees day^{-1}, respectively.

The BI increased during storage, and significant differences were observed over time and among accessions (Fig. 3C, Table 1). The accession with the fastest change in browning index over time was TG with a slope of 35.7 day^{-1}, followed by KH (31.8 day^{-1}), GF (30.1 day^{-1}) and SV (29.0 day^{-1}). Conversely, DL and PC had the smallest rate of change in browning index with linear slopes of 17.0 and 18.2 day^{-1}, respectively.

Correlation among the color parameters is shown in Table 2. A strong correlation of 0.96 ($P < 0.01$) was found between h° and BI, suggesting that hue is also a good indicator of the perceived browning intensity. Hue angle (h°) was selected as the color parameter to be used in the following studies to compare the correlation between browning and enzyme activities because it is a direct measurement of sample color and correlated well with the perceived browning severity, measured by BI. It was also used in combination with computer vision in a recent study (García et al., 2018) to characterize browning in lettuce.

The fourteen lettuce accessions evaluated in this study could be divided into six pedigree groups based on their lineage (Fig. 1). Significantly different browning potentials were observed among different pedigree groups. The PC group (PC, DL, GT) had the lowest browning potential, as demonstrated by slower rates of change in a* value, h°, and BI (Table 1). On the other hand, accessions in the TG group had high browning potential, with the highest in the group observed for KH, followed by SK. The accessions with one parent from a low browning pedigree group (PC group) and another from a high browning pedigree group (TG group) exhibited medium browning potential. This group included HD, RH, and TT (all contain both PC and TG in their ancestry), as well as SM (originates from a cross between PC and PI). The BI of these four accessions were closer to that of PC than that of TG. Lastly, GF and SV belong to yet another pedigree group, and both them showed similar patterns of browning. Thus, the browning potential appears to be strongly related to pedigree of accessions.

Seasonality plays a crucial role in the growth of produce and affects its shelf life significantly. Therefore, two more trials were performed in different seasons to verify the browning trends of the lettuce accessions. Results showed similar ranking in the browning potentials, where PC, DL, and GT had the lowest BI, whereas TG and KH exhibited the highest BI. Enzymatic activities and TPC, which will be presented in the next section, were measured in one of the three trials.

3.2. Change in PAL, POD, PPO activity and TPC during storage

Fig. 4 shows the change in the activities of three enzymes (PAL, PPO, and POD) and TPC during five days of storage. Among those parameters, the PAL activity (Fig. 4A) for most of the accessions was close to zero on day 0, except for PI. The PAL activity increased sharply during the first three days of storage. Accessions PI, TT, TG, KH, SK, and LB exhibited the highest PAL activity on day 5, while PC and DL showed the lowest PAL activity.

POD activity also increased during the five-day storage period (Fig. 4B), but at a much slower rate than PAL. Several accessions including KH, TG, and RH did not exhibit a significant increase in POD activity until day 3. Although differences in POD activities exist among accessions, the magnitude of the variation is small compared to PAL. Notably, the accession PI had very high POD activity on day 0, and it remained high throughout the five days of storage.

The PPO activities of most accessions (Fig. 4C) started high on day 0, fluctuated until reaching a maximum on day 4, and decreased thereafter. Two exceptions to this trend were DL and GF, whose PPO activities were relatively low on day 0 and increased by over 100% within three days. Among all accessions, SM and PC exhibited the highest mean PPO activity, while DL and GF showed the lowest mean PPO activity.

TPC varied greatly among different accessions on day 0 (Fig. 4D),

Table 1
Rates of change for colorimetric parameters including a*, b*, hue angle, and browning index (BI). The slopes were determined between Day 1 and Day 5. Accessions are sorted by their slopes for hue angles.

Accession	L* values		a* values		b* values		Hue angle (degrees)		Browning index	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
PC	−1.49a*	0.94	0.85f	0.95	1.10c	0.9	−3.79a	0.99	18.25e	0.93
DL	−1.67ab	0.98	0.81f	0.84	1.63bc	0.84	−4.21a	0.97	16.95e	0.96
GT	−2.37bc	0.98	1.89de	0.82	2.02b	0.92	−6.82b	0.99	22.72cd	0.95
LB	−1.93b	0.95	2.10d	0.83	1.29c	0.83	−6.96bc	0.99	21.39d	0.93
SM	−2.10bc	0.97	1.54e	0.85	1.94bc	0.88	−7.14bc	0.99	21.53d	0.94
PI	−2.68c	0.97	2.85bc	0.76	2.96a	0.90	−7.63c	0.98	22.24cd	0.91
TT	−1.99b	0.98	2.28cd	0.92	1.34c	0.96	−8.05c	0.99	23.67c	0.92
SK	−2.87cd	0.96	3.35b	0.88	2.16b	0.95	−9.20d	0.98	26.38bc	0.95
HD	−1.60ab	0.98	2.13d	0.92	1.26c	0.94	−9.26d	0.99	23.13cd	0.95
SV	−2.93cd	0.97	2.83bc	0.88	1.50bc	0.94	−10.31d	0.98	29.04b	0.93
RH	−2.42b	0.98	2.64c	0.93	1.77bc	0.91	−11.44e	0.98	25.72c	0.97
GF	−3.01cd	0.95	3.38ab	0.87	2.41ab	0.93	−11.68e	0.97	30.08b	0.97
KH	−2.99cd	0.98	3.46ab	0.83	2.48ab	0.94	−12.02e	0.99	31.84ab	0.94
TG	−3.29d	0.98	4.07a	0.88	1.79bc	0.84	−12.71e	0.99	35.69a	0.96

* Values with different letters show significant difference ($P < 0.01$) as per analysis of one-way covariance (ANCOVA) followed by Tukey's test.

Table 2
Correlation among color parameters in lettuce samples.

	L*	a*	b*	Hue angle
All accessions and storage time combined				
BI	−0.96*	0.92*	0.72*	−0.96*
L*		−0.88*	−0.82*	0.93*
a*			0.55*	−0.94*
b*				−0.72*

* Correlation is significant ($P < 0.01$).

ranging from 1.1 to 3.4 g per kg fresh weight. Accessions PI, followed by LB, SK, PC, DL, and TT had the highest TPC on day 0. TPC increased over time and ranged between 2.7 and 6.5 g per kg fresh weight on day 5. PI and LB showed the highest TPC values, followed by SK and TT, while HD, RH, SV, and DL were measured with the lowest TPC values.

3.3. Correlation between enzymes, TPC, and browning

Pearson correlation analysis was performed in two different ways to confirm the relationship between browning (represented by h°) and biochemical parameters. First, with all accessions combined, the temporal change of PAL, POD, PPO, and TPC was compared to changes in h°. As shown in Table 3, significant negative correlations were found between h° and PAL, POD, and TPC. Smaller h° values are indicative of greater browning, which suggests that an increase in browning is correlated to an increase in enzyme activities and phenolic content. The strongest correlation coefficient was found between PAL and h° ($r = -0.862$), followed by POD and TPC; PPO was not significantly correlated to h°.

Based on these results, linear regression was performed to model the impact of changing enzymatic activity and phenolic content on browning. The intercorrelation among PAL, POD, and TPC (Table 3) justified the use of simple linear regression instead of multilinear analysis.

As shown in Table 4, the linear model that included all 14 accessions fit best between browning and PAL, with the highest R^2 of 0.743, followed by POD (0.604) and TPC (0.425). A similar trend was observed for the models with specific pedigree groups.

In the second analysis, the accumulation of PAL, POD, PPO, and TPC during the five days of storage was calculated for each accession using the corresponding area under the curve (AUC) in Fig. 4. The correlation between AUC and the amount of browning (h°) on day 5 for each accession was determined. The accessions that belong to the same pedigree lines were also grouped together in the plot of browning with PAL (Fig. 5A) and POD (Fig. 5B). Specifically, the accessions from the PC pedigree group (PC, DL, and GT) showed low PAL AUC, medium POD AUC, and high h°, whereas those from TG group (TG and KH) exhibited high PAL and low POD AUC, and low h°. The mean PPO (Fig. 5C) and TPC (Fig. 5D) of the TG group were also higher than those of the PC group, although the difference was not statistically significant ($P > 0.05$). The accessions with both PC and TG in their ancestry (HD, RH, and TT) showed intermediate PAL, POD, and h° that fell in between those of the two parental families, but were in closer proximity to the PC group than to the TG group. The GF pedigree group was close to the TG group on the POD chart (Fig. 5B), but it exhibited relatively low PAL AUC (Fig. 5A), low PPO AUC (Fig. 5C) and TPC (Fig. 5D), and medium h°. Finally, accessions LB and PI were similar in terms of high PAL AUC (Fig. 5A), low PPO AUC (Fig. 5C), high TPC (Fig. 5D), and medium h°; however, PI showed exceptionally high POD AUC (Fig. 5B).

Correlation analyses were performed to determine the accumulative contribution of enzymes and phenolics on browning. Overall, the correlation was weak and dependent on the pedigree group. PAL AUC showed a moderate correlation to h° ($r = -0.439$), indicating a positive correlation to browning. Three pedigree groups (PC, TG, and PC x TG) appeared to follow this trend (Fig. 5A), whereas three other groups (GF, PI, and LB) did not follow the trend. POD AUC exhibited an unexpected positive correlation ($r = 0.491$) to h° (Fig. 5B), suggesting a negative relationship to browning. This trend was observed clearly in the TG, LB, andGFgroups (Fig. 5B), which showed that accessions with higher POD AUC tended to exhibit higher h° on Day 5. However, the PC and PC x TG group showed a weak correlation between POD and browning. No significant correlations were found between PPO AUC (Fig. 5C) or TPC AUC (Fig. 5D) and h° in any of the pedigree groups.

A correlation analysis was also performed between POD AUC/PAL AUC (abbreviated as P ratio) and h°. As shown in Fig. 6, the PC group was characterized by the greatest P ratio and high h°, whereas the TG group exhibited the lowest values. The PC x TG group was close to the PC group, which is consistent with the results in Fig. 5. The PI group and LB accession had relatively high P ratios and high h°, which was similar to the PC group. All the accessions showed that there was a positive trend between P ratio and h°, with the GF group deviating slightly from this trend. The correlation coefficient was 0.734 and 0.879 with and without GF group included, respectively.

4. Discussion

Breeding for browning-resistant cultivars of fresh produce has been the subject of intense research, given its major quality and economic impact on fresh-cut produce. Atkinson et al (2013a) evaluated the

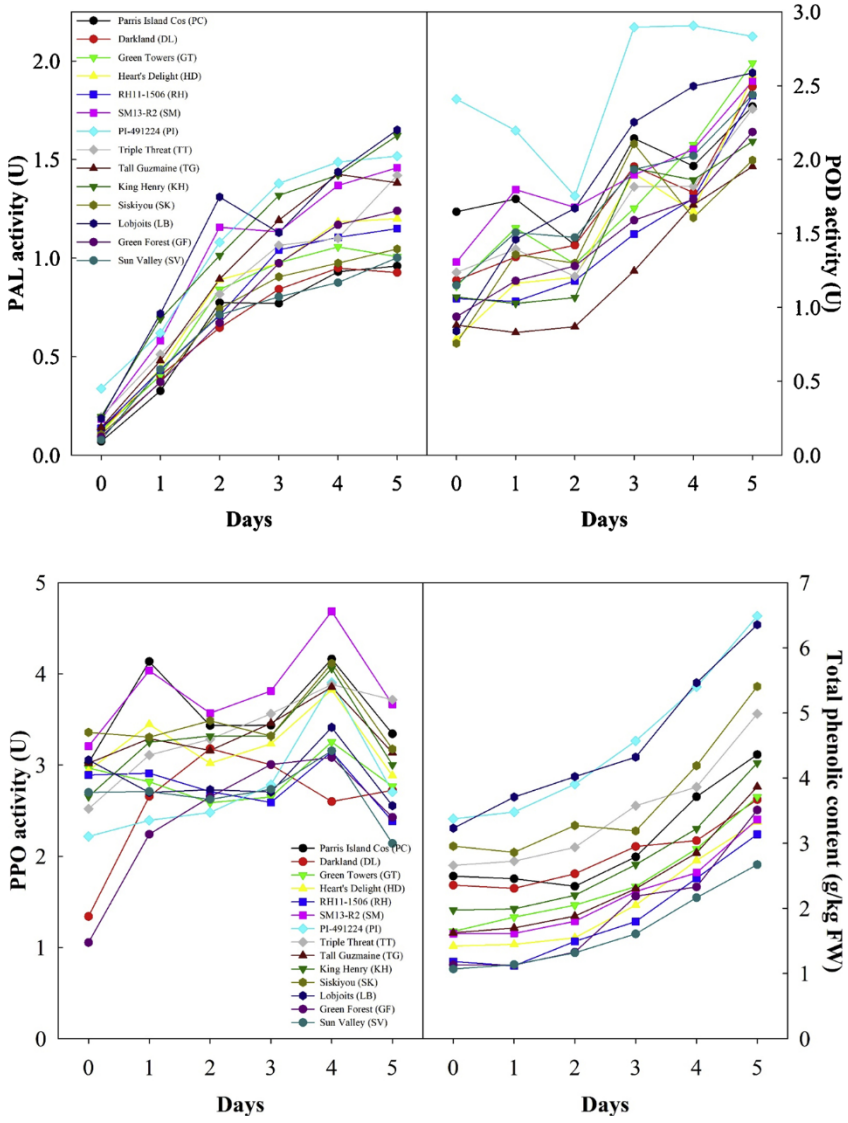

Fig. 4. Change in PAL, POD, and PPO activity and TPC during storage.

Table 3
Correlation between Hue angle, PAL, POD, PPO, and TPC over the five-day storage period.

	POD	PPO	TPC	Hue angle
All accessions and storage time combined				
PAL	0.67*	0.251*	0.529*	−0.862*
POD		0.105	0.672*	−0.777*
PPO			0.177*	−0.169*
TPC				−0.652*

* Correlation is significant ($P < 0.01$).

discoloration of cut lettuce among different lettuce types and accessions. An intra-specific linkage map of lettuce and a genetic analysis of postharvest discoloration traits was also created (Atkinson et al., 2013b). They suggested that the genetic basis for this phenotypic variation and the natural allelic variation could be exploited through breeding to develop discoloration resistant cultivars.

Lettuce accessions such as SK, GF, GT, and DL, have been characterized with a good shelf life, whereas those including KH and TT deteriorated severely during storage (Hayes and Liu, 2008). However, these analyses were performed under modified atmosphere packaging, which inflicts several disadvantages, as mentioned in the introduction. Therefore, this study aimed to find lettuce cultivars that possess low browning potential without the need for limited oxygen supply. This is a targeted study where most of the selected accessions were either assessed before, under different conditions, or are genetically related to those accessions. Specifically, GT and DL are single plant selections of PC, KH is a single plant selection of TG, and SV is the descendant of GF. SK, TT, HD, and RH accessions have at least one parent that originates from the PC or TG pedigree groups. Pedigree information allowed for a better understanding of the interaction between observed browning potential and genotype, and it can be used for more accurate selection of accessions with desirable traits in the future.

Our results (Fig. 5) demonstrated that browning and the underlying biochemical properties of lettuce are significantly influenced by lettuce genotype. The accessions belonging to different pedigree groups were found as distinct groups on the PAL/ h° (Fig. 5A) and POD/ h° (Fig. 5B) graphs. Compared to previous reports, the results from this study (Figs. 1 and 2) show differences in browning potentials between lettuce cultivars with ample oxygen supply, which should be considered when comparing these results to previous work that used MAP (Hayes and Liu, 2008). The GF accession showed good shelf life under MAP, but exhibited a relatively high degree of browning in this study. These results suggested that GF (and probably its descendant SV) had a high browning potential and a high respiration rate that depleted the oxygen

Table 4
Linear regressions between browning index and biochemical parameters. The regression model is written as $y = \beta_i x_i + \varepsilon_i$, where y denotes hue angle, x_i denotes one the three parameters [PAL (U), POD (U), and TPC (g/kg FW)], and β_i and ε_i are the slopes and intercepts for the linear equations, respectively.

Family*	PAL			POD			TPC		
	β	ε	R^2	β	ε	R^2	β	ε	R^2
All (14)	−31.6	124.9	0.743	−21.9	135.6	0.604	−33.4	124.8	0.425
PC (PC, DL, GT)	−26.3	123.2	0.820	−14.4	128.3	0.564	−35.4	129.8	0.556
TG (TG, KH, SK)	−34.8	125.1	0.822	−22.5	137.5	0.536	−33.8	125.8	0.468
PC x TG (HD, RH, TT) & PC x PI (SM)	−35.5	128.9	0.829	−25.1	142.9	0.684	−42.9	138.4	0.418
GF (GF, SV)	−45.5	127.6	0.880	−31.3	143.5	0.785	−69.7	136.6	0.790
PI (PI)	−25.2	124.2	0.871	−24.2	139.6	0.823	−85.9	139.8	0.942
LB (LB)	−19.9	121.2	0.777	−22.6	128.2	0.837	−59.0	138.2	0.937

* The abbreviations in parentheses show accessions that belong to the pedigree group. Definition of the abbreviations is listed in Fig. 1.

under MAP, reducing its browning levels (Martínez-Sánchez et al., 2011). Conversely, the severe deterioration observed for TT under MAP (Simko et al., 2012) was mostly due to disintegration of leaf tissue, not midribs (Simko et al., 2015). The other pedigree groups, such as PC and TG, showed comparable browning results with or without MAP, suggesting that the browning process of these pedigree groups were not affected by gas composition.

Given the known effects of enzymes and phenolics in lettuce browning (Choi et al., 2005; Gil, 2015; Saltveit, 2000), it was of interest to find out the correlation between biochemical parameters and h° of the lettuce samples in this study. Due to the significant effects of both time and accession on browning (result from two-way ANOVA; data not shown), we performed correlation analyses in two ways. In the first analysis, we investigated how the progression in browning (h°) correlated to the daily change in PAL, POD, PPO, and TPC. Results showed that browning correlated strongly and positively to PAL and POD (Table 3), which was in accordance with previous studies (Fukumoto et al., 2002; Hisaminato et al., 2001; López-Gálvez et al., 1996). However, the temporal change in TPC correlated positively to browning potential in our study, which was different from earlier reports that showed this correlation was insignificant because TPC was increased more slowly (Fukumoto et al., 2002; Mai and Glomb, 2013). One possible explanation for this difference in TPC results could be the processing methods; the lettuce samples in the aforementioned studies were treated with chlorinated water, whereas our study did not wash the lettuce samples.

In the second analysis, we investigated the accumulative effects of enzymes and phenols on the variation in browning severity among different accessions. To our knowledge, this type of analysis has been reported previously in a limited number of articles(Couture et al., 1993; Fukumoto et al., 2002; García et al., 2018). These results (Fig. 5) indicate there is a relationship between genetic variability and both browning and biochemical properties. The correlations among the investigated parameters were generally weak, in that different pedigree groups were found as distinct groups on the charts. The trends established between PAL (Fig. 5A) and browning were conformed by the PC,

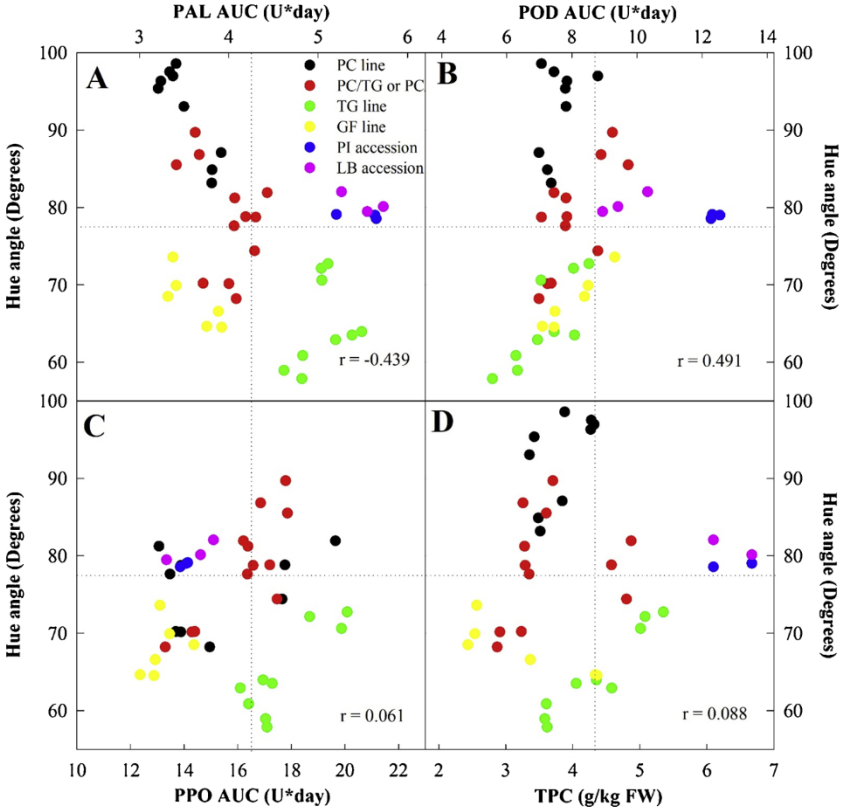

Fig. 5. Comparison of PAL, POD, PPO, TPC, and browning index among different accessions on Day 5. Accessions are grouped as follows: PC line (PC, DL, GT), PC/TG (HD, RH, TT), PC/PI (SM), TG line (TG, KH, SK), GF line (GF, SV), and PI and LB accessions. AUC refers to area under curve calculated from Fig. 4.

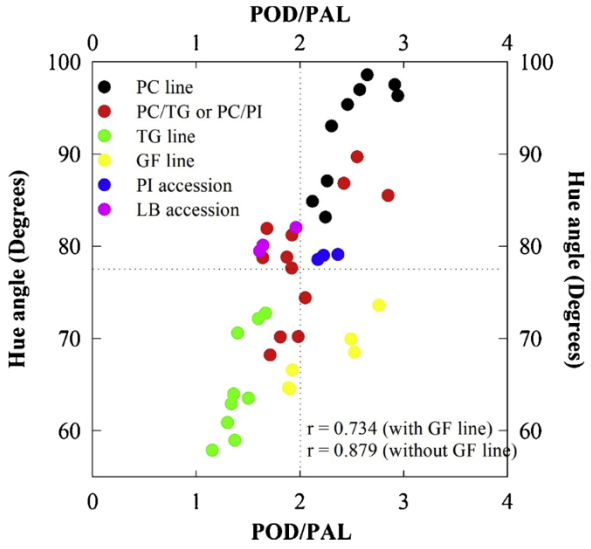

Fig. 6. Correlation between POD/PAL and browning index among different accessions on Day 5. Accessions are grouped as follows: PC line (PC, DL, GT), PC/TG (HD, RH, TT), PC/PI (SM), TG line (TG, KH, SK), GF line (GF, SV), and PI and LB accessions.

TG, and PC x TG groups, but other groups of lettuce accessions did not follow this trend. An unexpected negative trend was found between POD and browning among the accessions (Fig. 5B). The PPO (5C) and TPC (Fig. 5D) activities showed a weak correlation to browning when different accessions were compared. These results show the complexity of the browning process and the impracticality of explaining/predicting this process with only a single or a few factors. Some of the factors complicating these trends are discussed in the following paragraphs.

4.1. PAL

Among the biochemical parameters investigated in this study, PAL is the only parameter that had a positive correlation to browning in both correlation analyses. This finding confirmed that PAL has a major contribution to browning, as proposed previously by other researchers. Ke and Saltveit (1989) and López-Gálvez et al. (1996) reported the substantial increase in PAL activity immediately after cutting. López-Gálvez et al. (1996) Takahama reported the positive correlation between the slope of the PAL activity-time curve and browning score of cut iceberg lettuce. Tomás-Barberán et al. (1997) reported the inhibition of lettuce browning by washing with calcium chloride, a chemical known to suppress PAL activity.

4.2. POD

The seemingly contradictory correlations of POD to h° in the two analyses may be attributed to its controversial role in the browning process. On one hand, POD may participate directly in the oxidation of phenolics into colored products. Although the natural level of H_2O_2 is low in plant tissues, it may be replenished from PPO-catalyzed reactions (Richard-Forget and Gauillard, 1997). In addition, Takahama and Oniki (1997) proposed a POD/phenolics/ascorbate system that scavenges H_2O_2 in plant cells; they reported the formation of brown components in tobacco leaves by H_2O_2 and various phenolic compounds in the presence of POD. Takahama and Oniki (1997) further suggested that the H_2O_2 can be replenished by the autoxidation of excessive oxidized phenolics, which in turn facilitates the POD-catalyzed reaction. On the other hand, a higher POD activity is associated with a more rapid consumption of ascorbic acid (Eltelib et al., 2011), which can lead to elevated gene expression for enzymes, such as monodehydroascorbate reductase and dehydroascorbate reductase. These two enzymes play a key role in the recycling of ascorbic acid, increasing its content in plant tissues, and potentially inhibiting PPO- or POD-related browning (Fukumoto et al., 2002). These findings provide some possible explanations as to why the accessions with higher POD accumulation tended to show less browning.

4.3. Phenolic compounds

Similar to POD, TPC was positively correlated to the degree of browning on each day, but its accumulation at the end of storage did not show significant correlation to the final h° values among different accessions. The TPC assay measures the content of various phenolic compounds, of which only a few compounds have been identified with contribution to browning (Mai and Glomb, 2013). Tomás-Barberán et al. (1997) suggested that the accumulation of chlorogenic acid contributes to browning during storage. More recently, a metabolomic study has identified two phenolic compounds, caffeoylquinic acid and ferulic acid methyl ester, as positive and negative biomarkers for lettuce browning (García et al., 2018). Since the phenolic composition may vary greatly among different accessions of lettuce (DuPont et al., 2000), the amount of reactive phenolic compounds may not be represented accurately by their TPC values. This provides an explanation to the lack of correlation between TPC and browning among lettuce accessions. Measurement of individual phenolic compounds may provide a more accurate way for characterizing the browning potential in the future.- Nonetheless, the change in TPC over time showed significant correlation to the progression of browning, probably due to the simultaneous accumulation of both reactive (chlorogenic acid) and non-reactive phenolics, both of which account for the total phenolic content. Our results were also consistent with a previous study (Couture et al., 1993), which concluded that: (1) for one cultivar of lettuce, the TPC was positively correlated to browning; and (2) when different accessions were compared, those with a higher amount of TPC did not always exhibit more severe browning.

4.4. PPO

The PPO activity was not significantly correlated to browning in either of the correlation analyses, which was also reported previously (Fukumoto et al., 2002). The initial level of PPO observed on day 0 was relatively high compared to PAL and POD, and it either increased mildly or fluctuated during storage. Based on these observations, we hypothesize that PPO is not the rate-limiting factor of lettuce browning, and that its initial activity is sufficient for browning development. PPO is an important part of the plant defense system, and a considerable amount of PPO exists in its latent form under normal conditions; this latent PPO is converted to its active form when environmental stress is applied (Vaughn and Duke, 1984). A similar observation and hypothesis have been reported previously (Hisaminato et al., 2001).

4.5. Factors not investigated in this study

There are other factors whose roles in the browning process should be considered. The morphology and hardness of the lettuce ribs may affect the amount of mechanical injury that occurs during cutting, resulting in different levels of defensive responses associated with browning. In addition, the contents of certain active metabolites (JA, chlorogenic acid, certain fatty acids, etc.) may have a determinant effect on lettuce browning, as revealed by metabolomic analysis (García et al., 2018). The level of these metabolites are influenced by genetics and external factors, such as infection (Shoresh et al., 2005). The activities of other enzymes, such as PLD, also determine the progress of browning. In a relevant study, a total of sixteen enzymes such as isoflavone reductase and isoflavonoid regulator have been identified as potentially correlated to browning in apples (Lorenz et al., 2013). Moreover, the composition of storage proteins and amino acids also

determine the type and production rate of colored melanin products (Richard-Forget and Gauillard, 1997). The complex role of proteins and amino acids in browning may warrant a proteomic study on different lettuce accessions over various periods of storage. Finally, any change in physiological environment (pH, ionic strength, redox status, etc.) in response to cutting could have a significant impact on the enzymatic activity in vivo, thus affecting the progress of browning.

5. Conclusion

Fourteen romaine lettuce accessions were studied for their browning potentials and biochemical properties after cutting. A novel, computer-aided image analysis procedure was used to measure the change in h°, a colorimetric parameter that quantified the intensity of browning. Over five days of storage, the accessions in the TG (TG, SK, KH) and PC (PC, DL, GT) pedigree groups showed the highest and lowest levels of browning, respectively. Biochemical analyses showed a substantial increase in PAL activity after cutting, and a moderate increase in POD activity and TPC. PPO activity, on the other hand, increased to a lesser extent in eight accessions and decreased in six others. PAL, POD, and TPC increased with decreasing h° indicative of increasing browning levels), leading to a moderate to strong correlation among those parameters. By comparing all accessions, h° on day 5 had a moderate negative correlation to PAL accumulation but a positive correlation to POD accumulation. In addition, h° on day 5 showed no significant correlation to PPO or TPC accumulation. This is the first systematic study on the browning and biochemical indices of romaine lettuce from various pedigree groups using computer-enabled image analysis. Results from this study provide lettuce growers and breeders with guidance on the selection of accessions with limited browning potential, and it also supplies researchers in plant physiology and genetics with more comprehensive information on the roles of enzymes in lettuce browning.

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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.postharvbio.2019.110931.

References

Atkinson, L.D., Hilton, H.W., Pink, D.A., 2013a. A study of variation in the tendency for postharvest discoloration in a lettuce (L actuca sativa) diversity set. Int. J. Food Sci. Technol. 48, 801–807. https://doi.org/10.1111/ijfs.12030.

Atkinson, L.D., McHale, L.K., Truco, M.J., Hilton, H.W., Lynn, J., Schut, J.W., Michelmore, R.W., Hand, P., Pink, D.A., 2013b. An intra-specific linkage map of lettuce (Lactuca sativa) and genetic analysis of postharvest discolouration traits. Theor. Appl. Genet. 126, 2737–2752. https://doi.org/10.1007/s00122-013-2168-8.

Cantos, E., Espín, J.C., Tomás-Barberán, F.A., 2001. Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. J. Agric. Food Chem. 49, 322–330. https://doi.org/10.1021/jf000644q.

Choi, Y.-J., Tomás-Barberán, F.A., Saltveit, M.E., 2005. Wound-induced phenolic accumulation and browning in lettuce (Lactuca sativa L.) leaf tissue is reduced by exposure to n-alcohols. Postharvest Biol. Technol. 37, 47–55. https://doi.org/10.1016/j.postharvbio.2005.03.002.

Chua, D., Goh, K., Saftner, R., Bhagwat, A., 2008. Fresh-cut lettuce in modified atmosphere packages stored at improper temperatures supports enterohemorrhagic E. coli isolates to survive gastric acid challenge. J. Food Sci. 73, M148–M153. https://doi.org/10.1111/j.1750-3841.2008.00698.x.

Couture, R., Cantwell, M., Ke, D., Saltveit, M., 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. HortScience 28, 723–725. https://doi.org/10.21273/HORTSCI.28.7.723.

Dixon, R.A., Achnine, L., Kota, P., Liu, C.J., Reddy, M.S., Wang, L., 2002. The phenylpropanoid pathway and plant defence—a genomics perspective. Mol. Plant Pathol. 3, 371–390. https://doi.org/10.1046/j.1364-3703.2002.00131.x.

DuPont, M.S., Mondin, Z., Williamson, G., Price, K.R., 2000. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. J. Agric. Food Chem. 48, 3957–3964. https://doi.org/10.1021/jf0002387.

Eltelib, H.A., Badejo, A.A., Fujikawa, Y., Esaka, M., 2011. Gene expression of monodehydroascorbate reductase and dehydroascorbate reductase during fruit ripening and in response to environmental stresses in acerola (Malpighia glabra). J. Plant Physiol. 168, 619–627. https://doi.org/10.1016/j.jplph.2010.09.003.

Fukumoto, L.R., Toivonen, P.M., Delaquis, P.J., 2002. Effect of wash water temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce photosynthetic and vascular tissues. J. Agric. Food Chem. 50, 4503–4511. https://doi.org/10.1021/jf011415m.

García, C.J., Gil, M.I., Tomas-Barberan, F.A., 2018. LC–MS untargeted metabolomics reveals early biomarkers to predict browning of fresh-cut lettuce. Postharvest Biol. Technol. 146, 9–17. https://doi.org/10.1016/j.postharvbio.2018.07.011.

Gil, M., 2015. Preharvest factors and fresh-cut quality of leafy vegetables. III International Conference on Fresh-Cut Produce: Maintaining Quality and Safety 1141, 57–64. https://doi.org/10.17660/ActaHortic.2016.1141.6.

Hayes, R.J., Liu, Y.-B., 2008. Genetic variation for shelf-life of salad-cut lettuce in modified-atmosphere environments. J. Am. Soc. Hortic. Sci. 133, 228–233. https://doi.org/10.21273/JASHS.133.2.228.

Hisaminato, H., Murata, M., Homma, S., 2001. Relationship between the enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibitors of polyphenol biosynthesis. Biosci. Biotechnol. Biochem. 65, 1016–1021. https://doi.org/10.1271/bbb.65.1016.

Hunter, P.J., Atkinson, L.D., Vickers, L., Lignou, S., Oruna-Concha, M.J., Pink, D., Hand, P., Barker, G., Wagstaff, C., Monaghan, J.M., 2017. Oxidative discolouration in whole-head and cut lettuce: biochemical and environmental influences on a complex phenotype and potential breeding strategies to improve shelf-life. Euphytica 213, 180. https://doi.org/10.1007/s10681-017-1964-7.

Hyodo, H., Kuroda, H., Yang, S.F., 1978. Induction of phenylalanine ammonia-lyase and increase in phenolics in lettuce leaves in relation to the development of russet spotting caused by ethylene. Plant Physiol. 62, 31–35. https://doi.org/10.1104/pp.62.1.31.

Ke, D., Saltveit Jr, M.E., 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. Physiol. Plant. 76, 412–418. https://doi.org/10.1111/j.1399-3054.1989.tb06212.x.

Kim, J.G., Luo, Y., Tao, Y., Saftner, R.A., Gross, K.C., 2005a. Effect of initial oxygen concentration and film oxygen transmission rate on the quality of fresh-cut romaine lettuce. J. Sci. Food Agric. 85, 1622–1630. https://doi.org/10.1002/jsfa.2158.

Kim, J.G., Luo, Y.G., Saftner, R.A., Gross, K.C., 2005b. Delayed modified atmosphere packaging of fresh-cut romaine lettuce: effects on quality maintenance and shelf-life. J. Am. Soc. Hortic. Sci. 130, 116–123. https://doi.org/10.21273/JASHS.130.1.116.

Landi, M., Degl'Innocenti, E., Guglielminetti, L., Guidi, L., 2013. Role of ascorbic acid in the inhibition of polyphenol oxidase and the prevention of browning in different browning-sensitive Lactuca sativa var. capitata (L.) and Eruca sativa (Mill.) stored as fresh-cut produce. J. Sci. Food Agric. 93, 1814–1819. https://doi.org/10.1002/jsfa.5969.

LaVigne, A.W., 2017. The Next Farm Bill: Technology & Innovation in Specialty Crops. American Seed Trade Association. Retrieved athttps://www.betterseed.org/wp-content/uploads/Andrew-LaVigne-House-Ag-testimony-7-12-17.pdfDOI unavailable.

Lester, G.E., Lewers, K.S., Medina, M.B., Saftner, R.A., 2012. Comparative analysis of strawberry total phenolics via Fast Blue BB vs. Folin–ciocalteu: assay interference by ascorbic acid. J. Food Compos. Anal. 27, 102–107. https://doi.org/10.1016/j.jfca.2012.05.003.

López-Gálvez, G., Saltveit, M., Cantwell, M., 1996. Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuces. Postharvest Biol. Technol. 9, 223–233. https://doi.org/10.1016/S0925-5214(96)00050-6.

Lorenz, G., Masuero, D., Vrhovsek, U., Velasco, R., Costa, F., Farneti, B., Costa, G., Di Guardo, M., Tadiello, A., 2013. Multidisciplinary approach provides novel insight about fruit flesh browning physiology in apple (Malus× domestica Borkh.). III International Symposium on Molecular Markers in Horticulture 1100, 161–166. https://doi.org/10.17660/ActaHortic.2015.1100.25.

Luna, M.C., Tudela, J.A., Tomás-Barberán, F.A., Gil, M.I., 2016. Modified atmosphere (MA) prevents browning of fresh-cut romaine lettuce through multi-target effects related to phenolic metabolism. Postharvest Biol. Technol. 119, 84–93. https://doi.

org/10.1016/j.postharvbio.2016.05.001.
Luo, Y., 2007. Wash operation affect water quality and packaged fresh-cut romaine lettuce quality and microbial growth. HortScience 42, 1413–1419. https://doi.org/10.21273/HORTSCI.42.6.1413.
Mai, F., Glomb, M.A., 2013. Isolation of phenolic compounds from iceberg lettuce and impact on enzymatic browning. J. Agric. Food Chem. 61, 2868–2874. https://doi.org/10.1021/jf305182u.
Martínez-Sánchez, A., Tudela, J.A., Luna, C., Allende, A., Gil, M.I., 2011. Low oxygen levels and light exposure affect quality of fresh-cut Romaine lettuce. Postharvest Biol. Technol. 59, 34–42. https://doi.org/10.1016/j.postharvbio.2010.07.005.
Murata, M., Tanaka, E., Minoura, E., Homma, S., 2004. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement on sensory evaluation during storage. Biosci. Biotechnol. Biochem. 68, 501–507. https://doi.org/10.1271/bbb.68.501.
Richard-Forget, F.C., Gauillard, F.A., 1997. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (Pyrus communis cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. J. Agric. Food Chem. 45, 2472–2476. https://doi.org/10.1021/jf970042f.
Saltveit, M.E., 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. Postharvest Biol. Technol. 21, 61–69. https://doi.org/10.1016/S0925-5214(00)00165-4.
Sharma, M., Lakshman, S., Ferguson, S., Ingram, D.T., Luo, Y., Patel, J., 2011. Effect of modified atmosphere packaging on the persistence and expression of virulence factors of Escherichia coli O157: H7 on shredded iceberg lettuce. J. Food Prot. 74, 718–726. https://doi.org/10.4315/0362-028X.JFP-10-427.
Shoresh, M., Yedidia, I., Chet, I., 2005. Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by Trichoderma asperellum T203. Phytopathology 95, 76–84. https://doi.org/10.1094/PHYTO-95-0076.
Simko, I., Hayes, R., Mou, B., Mccreight, J., 2014. Yield gains in leafy vegetables. In: Smith, S., Diers, B., Specht, J., Carver, B. (Eds.), Yield Gains in Major U.S. Field Crops. American Society of Agronomy, Inc., Madison, WI, USA, pp. 53–86. https://doi.org/10.2135/cssaspecpub33.
Simko, I., Hayes, R.J., Kramer, M., 2012. Computing integrated ratings from heterogeneous phenotypic assessments: a case study of lettuce postharvest quality and downy mildew resistance. Crop Sci. 52, 2131–2142. https://doi.org/10.2135/cropsci2012.02.0111.
Simko, I., Hayes, R.J., Truco, M.-J., Michelmore, R.W., Antonise, R., Massoudi, M., 2018. Molecular markers reliably predict post-harvest deterioration of fresh-cut lettuce in modified atmosphere packaging. Hortic. Res. 5, 21. https://doi.org/10.1038/s41438-018-0022-5.
Simko, I., Jimenez-Berni, J.A., Furbank, R.T., 2015. Detection of decay in fresh-cut lettuce using hyperspectral imaging and chlorophyll fluorescence imaging. Postharvest Biol. Technol. 106, 44–52. https://doi.org/10.1016/j.postharvbio.2015.04.007.
Takahama, U., Oniki, T.A., 1997. peroxidase/phenolics/ascorbate system can scavenge hydrogen peroxide in plant cells. Physiologia Plantarum. 101 (December (4)), 845–852. https://doi.org/10.1111/j.1399-3054.1997.tb01072.x.
Tomás-Barberán, F.A., Gil, M.I., Castaner, M., Artés, F., Saltveit, M.E., 1997. Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. J. Agric. Food Chem. 45, 583–589. https://doi.org/10.1021/jf960478f.
Tudela, J.A., Hernández, N., Pérez-Vicente, A., Gil, M.I., 2016. Comprehensive evaluation of different storage conditions for the varietal screening of lettuce for fresh-cut performance. Postharvest Biol. Technol. 120, 36–44. https://doi.org/10.1016/j.postharvbio.2016.05.009.
Vaughn, K.C., Duke, S.O., 1984. Function of polyphenol oxidase in higher plants. Physiol. Plant. 60, 106–112. https://doi.org/10.1111/j.1399-3054.1984.tb04258.x.
Zhan, L., Li, Y., Hu, J., Pang, L., Fan, H., 2012. Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light. Innov. Food Sci. Emerg. Technol. 14, 70–76. https://doi.org/10.1016/j.ifset.2012.02.004.