Visible light as a new tool to maintain fresh-cut lettuce post-harvest quality

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\textbf{A B S T R A C T}

Fresh-cut lettuces are susceptible to tissue browning and quality deterioration during post-harvest storage, even if they are kept in cold temperature. In this study we tried to counteract these undesirable physiological disorders by testing either storage under continuous light or after short treatments (2 d) with intermittent light (2 h on/2 h off) followed by storage in darkness. Two light intensities, 50 and 150 μmol m\textsuperscript{-2} s\textsuperscript{-1}, were studied. Continuous light (50 or 150 μmol m\textsuperscript{-2} s\textsuperscript{-1}) significantly inhibited tissue browning but stimulated dehydration. However, intermittent light during 2 days minimized browning and water loss and showed a global positive residual physiological change during the following 5 d of storage in darkness. All light treatment maintained the photosynthetic capacity of fresh cut lettuces excepting for high continuous light (150C). The photosystem II efficiency was negatively affected by both the continuous and intermittent light at 150 μmol m\textsuperscript{-2} s\textsuperscript{-1} but not by the moderate intermittent light (50 μmol m\textsuperscript{-2} s\textsuperscript{-1}). Finally, among the overall conditions tested, the short treatment (2 d) of fresh-cut lettuce by intermittent moderate level light (50 μmol m\textsuperscript{-2} s\textsuperscript{-1}) followed by storage in darkness appeared to be the best compromise. Although not yet ideal, this treatment could maintained the product quality by reducing browning, minimizing weight loss and respiration and also keeping high level of photosynthetic capacity. Future studies in this context of visible light based post-harvest treatments are consequently promising.

1. Introduction

Fresh-cut fruit and vegetables belong to the market segment that had shown the highest economical progression in the food industry history (Martin-Bellosol and Soliva-fortuny, 2011) and has continued to grow up to 5–10% per year during the past decade. Salads represent 50% of this segment and tissue browning is the most common and serious disorder detected and rejected by consumers.

Cut-edge browning results from an oxidation of the phenolic compounds present in the samples or neosynthesised after cutting mainly by polyphenoloxidase (PPO). The operations involved in the processing of fresh-cut lettuce such as cutting and drying cause tissue damage leading to a rapid quality deterioration and shelf-life reduction. Chemical treatments such as reducing agents or enzyme inhibitors have been studied to control the phenolic metabolism that leads to browning. However, the industry still needs a strategy to prevent browning without the use of chemical agents. Several physical methods have been proposed to extend the shelf-life such as modified atmosphere packaging (MAP) (Charles et al., 2005), heat treatments (Campos-Vargas et al., 2005; Dijoua et al., 2009; Salman et al., 2008, 2007) or UV light application (Allende et al., 2006; Charles et al., 2013).

Visible light exposure represents a novel approach, environmental-friendly, that can be used to preserve the overall quality of fresh-produce. It is well known that in plants, darkness induces the expression of genes implicated in chlorophyll, protein and chloroplast degradation and an increase of the reactive species of oxygen (Wada and Ishida, 2009). However, in many cases, light is not controlled during post-harvest storage and products are commercially stored in darkness, which induces senescence and accelerates this process significantly. Light exposure can retard tissue browning of fresh-cut romaine lettuces (Zhan et al., 2012), fresh-cut celery (Zhan et al., 2013c) and delayed the yellowing of broccoli (Bücht et al., 2011).

Light has also a positive effect on nutritional quality. Continuous light (around 35 μmol m\textsuperscript{-2} s\textsuperscript{-1}) maintained soluble sugars and ascorbic acid in fresh-cut romaine lettuce (Zhan et al., 2013a). In spinach leaves, the endogenous pool of some vitamins (such as ascorbic acid, folate,…) showed higher values when leaves where stored under visible light than in the dark (Lester et al., 2010). Light could enhance ascorbic acid synthesis due to the increase of the photosynthetic capacity that induce the availability of soluble carbohydrates, especially glucose, enabling them to contribute to the control of the ascorbic acid pool size (Toledo et al., 2003).

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Recent works studied the effect of light applied in a not continuous way. Light pulses of 30 μmol m$^{-2}$ s$^{-1}$ can be used to extend post-harvest shelf-life of spinach leaves (Gergoff Grozeff et al., 2013). Daily exposure for 2 h to 30–37 μmol m$^{-2}$ s$^{-1}$ of light was sufficient to delay postharvest senescence of basil leaves and suppress chlorophyll and protein loss (Costa et al., 2013). In Lamb’s lettuces, intermittent low intensity light cycles increased only partially photosynthesis, while the metabolism of the green tissues is still able to provide carbon moieties for the synthesis of bioactive molecules involved in delaying senescence (Braidot et al., 2014).

However, light could also decreased the quality of fresh-cut leek (Ayala et al., 2009), asparagus (Sanz et al., 2009) and cauliflower (Olarte et al., 2009). Thus, the influence of light on fresh-cut vegetables during storage is controversial, since both positive and negative effects on shelf-life and quality have been observed. Furthermore, the effects of postharvest illumination on chloroplast have not been extensively investigated. Most of the time, light is applied continuously and with medium intensity (around 30 μmol m$^{-2}$ s$^{-1}$). This study wants first to investigate the effect of two light intensities: a medium and a high. Then, the impact of light cycle treatments will be analyzed by applying continuous and intermittent light and by studying the effect on the storage of fresh-cut lettuces. The objectives of this study were to characterize the effects of light on browning and on electron transport chain in Calvin cycle in lettuces. The objectives of this study were to characterize the effects of light on browning and whether the photosynthetic parameters from chlorophyll a fluorescence and gas exchanges are permanently influenced by light in post-harvest and whether they are also good senescence markers.

2. Materials and methods

2.1. Plant materials and fresh-cut process

Butterhead lettuces (Lactuca sativa L.) were purchased at a local market in Avignon, France, and were immediately transported to the laboratory. The outer leaves, the core and the upper part of lettuces were removed and leaves of uniform size and color (inner leaves) were selected. The leaves were washed in chlorinated water (80 mg L, pH = 7.4) for 10 min, rinsed with tap water for 5 min and then dried on blotting paper. Four leaves were packaged in OPP film (Oriented polypropylene film, CFS, France) of 40 × 30 cm and 40 μm thickness (po2 = 2.43 × 10$^{-16}$ mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$). Three bags were used per treatment and per time.

2.2. Visible light treatments

Packaged lettuce leaves were stored at 6°C in standard climatic chambers (Sanyo MLR-351-H, Panasonic, USA) equipped with cool white fluorescent lamps (Mitsubishi OSRAM 44w s/37), according to the following conditions (Fig. 1): 7 d of darkness (control); 7 d of continuous visible light at 150 μmol m$^{-2}$ s$^{-1}$ (150C) or 50 μmol m$^{-2}$ s$^{-1}$ (50C); 2 d of intermittent light (2 h on/2 h off) at either 150 μmol m$^{-2}$ s$^{-1}$ (50LD) or 50 μmol m$^{-2}$ s$^{-1}$ (50LD) followed by 5 d of storage in darkness. The samples were arranged on the shelves in order to receive similar light irradiance. The photosynthetic active radiation (PAR) was performed by a radiometer (PMA2100, Solar Light).

2.3. Cut-edge browning, weight loss and gas content

Visual quality was scored by a four member trained panel. The quality was evaluated considering the color of the cut-edges and according to a five points hedonic scale running from 1 = good quality (absence of cut-edge browning) to 5 = severe browning. In addition, color was assessed by a colorimeter CR-400 (Konica Minolta, Japan) under standard illuminant D65. The percentage of weight loss was calculated by deducting sample weighting of each day from J0. O2 and CO2 content inside each lettuce packages were measured by a gas analyzer (Checkmate 9900, PBI Dansensor, France).

2.4. Net photosynthesis and chlorophyll fluorescence

Net photosynthesis (Pn) was measured using a portable photosynthetic analyzer (LI-Cor, Lincoln, NE, USA) equipped with a leaf dedicated chamber. Measurements were done under various light intensity, 400 ppm fixed CO2, ambient temperature and humidity. Net photosynthesis (Pn) was deduced under light as a result of gross photosynthesis (Pg) and respiration (Pn = Pg − R). The LI-Cor was also used to determine the lettuce light compensation point.

To assess the behavior of photosystem II (PSII), chlorophyll a fluorescence (expressed in relative units) was measured with a portable Handy-PEA (Hansatech, Kings Lynn, UK). Prior to measurement, lettuce leaves were dark-adapted for 30 min. JIP-test analysis was performed by the apparatus according to the energy flux theory of the thylakoid membrane to determine several indices (Strasserf and Srivastava, 1995). In this study we focalized on Fv/Fm (maximal photochemical efficiency of PSII) and Pi (Performance Index) for all conditions.

2.5. Statistical analysis

A total of three packaging were analyzed for each day and each condition tested and all the analyses were performed on each leaves (four leaves per bag). Thus, data represent mean values of three replicates (n = 12). Error bars represents standard error. Statistical significance of the data was determined according to the Kruskal-Wallis test followed by Dunn’s comparative post hoc method (p = 0.05). Significant groups of data are indicated on the figures by small letters.

3. Results

3.1. Cut-edge browning

Lettuces kept in darkness showed the strongest cut-edge browning (Fig. 2A). A score of 4 (browning initiation) was obtained after 4 d and it quickly reached a score of 3 (moderate browning) under the limit of marketability, at the end of storage. Samples stored under light conditions showed a better visual quality in terms of browning. Under continuous light and whatever the intensity (150C and 50C), no browning was observed during all the storage period. In addition, there was no browning with intermittent 150 μmol m$^{-2}$ s$^{-1}$ light (150 LD). Finally, a slight cut-edge browning was observed at day 7 with the 50LD treatment.

Fig. 1. Light treatments of fresh-cut lettuces.
The cut-edge browning was also measured with a chromameter and expressed as Chroma and Hue values (Fig. 2B and C). These data confirm that continuous light and high light intensity best maintain the sample cut-edge color. After 7 d of storage, samples kept in darkness showed the higher color modification indicating their darkening (Chroma) and browning (Hue). The 50LD treatment showed again an intermediate effect.

### 3.2. Fresh weight loss

Fresh weight loss is widely used to evaluate the freshness of fresh-cut lettuces (Fig. 3). Storage under continuous light during 7 d dramatically increased leaf weight loss up to 30% (150C and 50C). 2 d intermittent treatments (especially 50LD) appeared to slightly affect dehydration that stayed under 10%. The control (storage in darkness) maintained the weight loss under the 5% recommendation.

### 3.3. Light compensation point, photosynthesis and gas change

The light compensation point is the light intensity where the rate of photosynthesis matches the rate of respiration. For the lettuce leaves it was around 30 mmol m$^{-2}$ s$^{-1}$ (Fig. 4). To ensure a minimum of net photosynthesis, we used this value to define the low intensity light treatments as 50 mmol m$^{-2}$ s$^{-1}$ (50C and 50LD).

At the end of storage (day 7), the response of the net photosynthesis to the various storage conditions was evaluated (Fig. 5). Photosynthesis was measured under 50, 150 and saturating 1500 mmol m$^{-2}$ s$^{-1}$ light intensity. As expected, the photosynthesis rate was proportional to the intensity of the light applied. Excepted for the continuous high light treatment (150C), all conditions (including darkness to a lesser extend) showed a good level of photosynthesis at the end of the storage period. However, the negative values displayed with 150C treatment indicated that these samples underwent a much greater respiration rate than photosynthesis. Finally, the medium light intensity treatments (50C and 50LD) appeared to best preserve the photosynthetic activity of fresh-cut lettuce.

Changes of O$_2$ and CO$_2$ levels in packaging of lettuces are shown in Fig. 6. As expected, in darkness, O$_2$ content decreased and CO$_2$ content increased more than all the others conditions, due to respiration. These modifications where particularly intense during the first 2 d of storage, and then stabilized due to gas equilibrium according to the film permeability. Under continuous light treatments and whatever the intensity (50 and 150C), O$_2$ and CO$_2$ contents were stable and around 20 and 0%, respectively. Concerning the intermittent light treatments (50 and 150LD), O$_2$ and CO$_2$ contents only started to change after the 2 d treatments and until 4 d. This is an expected respiration behavior (CO$_2$ increased and O$_2$ decreased) since these samples were stored back in darkness from day 2. Then, both contents (O$_2$ and CO$_2$) stabilized at a middle level between the values of “darkness” and “continuous light” treatments.

### 3.4. Chlorophyll a fluorescence

The maximal photochemical efficiency of the photosystem II (Fv/Fm) and its Performance Index (Pi) were measured (Fig. 7A and B). Globally, Fv/Fm and Pi remained stable for lettuce samples kept in darkness or exposed to medium light intensity (50LD and 50C). However, high light treatment either continuous during 7 d or intermittent during 2 d (150C and 150LD) rapidly induced a significant decrease of the both parameters during the first 2 d. Then, Fv/Fm and Pi remained low until the end of the experiment. When looking to the overall treatments, it was not possible to discriminate between intermittent and continuous light.
4. Discussion

4.1. Light influence on cut-edge browning and water loss

We observed that high (150 μmol m⁻² s⁻¹) and medium (50 μmol m⁻² s⁻¹) continuous light intensity inhibited the cut-edge browning of fresh-cut lettuces during a 7 d storage period (Fig. 2). These results are in agreement with Zhan et al. who reported that continuous light around 35 μmol m⁻² s⁻¹ intensity decreased the browning of fresh-cut romaine lettuce and fresh-cut celery (Zhan et al., 2012, 2013c). In this study, we also experimented 2 d intermittent light treatments (2 h on/2 h off) followed by a 5 d storage in darkness (LD). The 150 LD treatment also inhibited the cut-edge browning although the salads are placed in the dark for 5 days. This result demonstrated that the effect of light was residual and was maintained in the dark. Interestingly, the lettuce treated by the 50 μmol m⁻² s⁻¹ intermittent light (50 LD) showed a slight cut-edge browning only at the end of storage (days 7). Thus, the inhibition of browning increased as the light intensity increased and the residual effect was also dependent of the light intensity. The effect of light intensity was described in model systems by Manzocco et al. (Manzocco et al., 2009). However the mechanism of action of light was not known precisely. Manzocco et al. said that visible light could inactivate polyphenol oxidase (PPO) activity due to nonreversible structural changes. Zhan et al. (2013b) have proposed that samples exposed to light could contain more ascorbic acid than those stored in darkness and inhibit the catalytic action of PPO by...
allowing enough CO₂ supply as required with an intense photosynthetic activity, the low CO₂ permeability of the packaging can not be able to inhibit PPO neosynthesis following cutting, (2) it can alter the pathways of synthesis of phenolic compounds and reduce the amounts of oxidizable phenols, and (3) it may also contribute to reducing browning due to a slight dehydration of the surface of the cuts.

This latter hypothesis must not be ruled out because in the present study, despite the positive effect of continuous light (50C and 150C) on cut-edge browning, it also induced a high water loss in comparison to the darkness storage that showed the lowest dehydration (Fig. 3). It is well known that during the growth of plants, light induced a stomatal opening in order to enhance CO₂ uptake by leaf for photosynthesis, and in others conditions (darkness, water deficit) stomata must close. Few studies have described stomatal conductances changes after fresh-cut process. Martínez-Sánchez et al. (2011) have reported that light exposure during storage increased stomatal aperture and consequently water loss. Our data are in line with this: under continuous light, dehydration increased continuously (50C and 150C, Fig. 3). Although temperature was maintained at 6 °C along the whole experiment, continuous light induced a quite small but significant temperature increase inside the leaves, leading to an hydric potential imbalance between leaf and atmosphere and the consequent dehydration. However, under intermittent light (50LD and 150LD), the dehydration rate was maximum during the first 2 d (corresponding to the light treatment) and then, during storage in darkness, the water loss stabilized and was not significantly different with darkness condition at the end of storage.

Thus, it is necessary to find a compromise between inhibition of browning and water loss due to light, and intermittent visible light treatment seems to be an effective alternative solution.

4.2. Light effect on photosynthesis

The fresh-cut lettuces net photosynthesis has been evaluated at the end of the 7 d storage period (Fig. 5). The photosynthetic capacity is a good indicator of senescence since it reflects directly the Rubisco capacity to fix CO₂. It is known that the amount of Rubisco decreases rapidly in the early phase of senescence and it has been previously demonstrated that light decreased Rubisco degradation (and other protein) and maintained chloroplasts integrity (Wada and Ishida, 2009).

This is consistent with our photosynthesis measurements under 50 μmol m⁻² s⁻¹ light treatments were able to maintain high degree of CO₂ fixation in comparison with the control (darkness) (Fig. 5). This is especially true when we analyzed the data obtained under 1500 μmol m⁻² s⁻¹ radiance, the maximal photosynthesis. Concerning the 150C treatment, we can correlate the near lack of photosynthetic metabolism to the low CO₂ content inside the packaging (Fig. 6). Indeed, the low CO₂ permeability of the packaging couldn’t be able to allow enough CO₂ supply as required with an intense photosynthetic intensity and samples are stored under the CO₂ compensation point. These conditions, in confined environment, are very favorable to the Melzer’s reaction and to the production of reactive species of oxygen and the consequent chloroplast degradation (Sallanon et al., 1997). The effect of dehydration appeared to be less pronounced, since leaves stored under 50C lose more than 20% of water and have the greatest photosynthetic capacity.

It is also interesting to see that exposing lettuce to intermittent light during two days (50LD and 150LD) was enough to maintain a high photosynthetic capacity until the end of the following 5 d storage period in darkness. In this case, the samples seem to display a protective residual effect of the photosynthesis capacity. Moreover, modifications of the daily light circadian cycle can also maintain plant integrity (Darwish et al., 2015a,b). Darwish et al., have demonstrated that a short duration cycling of light/darkness applied for a few days increased tobacco leaves antioxidant activities, and this effect was maintained several weeks after returning in normal light conditions. Recent studies have also reported that maintenance of the daily cycling of light and dark periods during postharvest may slow the decline of green leafy vegetables, preserve the tissue integrity and improve the nutritional content of plants (Liu et al., 2015).

To complement the above findings, our monitoring data of the gas content inside the lettuce packages are also supporting the moderate intermittent light treatments (Fig. 6). As expected, sample stored in darkness during the overall experiment displayed a high respiration behavior that is characteristic of the accelerated senescence. On the opposite, samples exposed to continuous light during 7 d (50C and 150C) displayed no (or even very low) modification of the gas content. Though light exposure promote respiration activity, the photosynthetic activity consumes the CO₂ produced by the respiration and CO₂ content remains low. Finally, regarding intermittent light, when the 50LD and 150LD samples were brought back in darkness (day 2, Fig. 6), they displayed a low modification of both CO₂ and O₂ content in comparison to the control (darkness). Moreover, 50LD gave the smaller gas content modulation, thus it tends to reduce the respiration metabolism. This finding suggested again that the first 2 d of moderate intermittent light (50LD) appeared as an interesting compromise between darkness storage and other light treatments tested.

4.3. Effect of light treatments on chlorophyll fluorescence parameters

In post-harvest, among the non-invasive and highly sensitive tools for evaluating plant stress under different conditions, chlorophyll a fluorescence measurement is a widely used method (Strasser and Srivastava, 1995). In this study, we analyzed the maximum quantum yield from PSII (Fv/Fm) and its performance index (Pi) as derived from chlorophyll a fluorescence measurement. Both parameters displayed a rapid decrease in response to the high light treatments (150LD and 150C, Fig. 7) regardless to the duration (7 d or 2 d) and the type of treatment (continuous or intermittent light). This indicated that these fluorescence parameters are good indicator of the perception of a light stress by the plant and of the photoinhibition process, as it was described by numerous authors (Lawlor and Cornic, 2002). The chloroplast electron transport chain was affected by the high light intensity (150 μmol m⁻² s⁻¹) and in the case of intermittent light treatment, 5 days in darkness makes it impossible to regain normal functioning. Toivonen et al. (Toivonen, 1992) have shown that a decrease in variable fluorescence (Fv) in broccoli florets was highly correlated with a reduction in respiration rate and ascorbic acid content during 4–24 d of storage at 1 °C, suggesting that fluorescence may be a reliable indicator of broccoli senescence. Ferrante et al. (Ferrante and Maggiori, 2007) have reported that Fv/Fm measurements were correlated to the increase of the storage time. In this study, and particularly with 150 continuous and intermittent and darkness treatments, the decrease in Fv/Fm and Pi was not correlated with photosynthetic capacity. Thus, it was not possible to systemically associate the decrease of Fv/Fm and Pi to the dehydration of the samples. Two hypothesis can be formulated: in the case of the 150C samples (highly dehydrated, and showing no photosynthetic capacity), the light harvesting complex itself was reversibly damaged. This typically occurs when the CO₂ assimilation is overwhelmed by the light absorption (Tozzi et al., 2013). Concerning the 150LD samples (showing low dehydration and still significant photosynthetic capacity at the end of storage), the protective mechanism of non-photochemical quenching could be enhanced (Giovagnetti and Ruban, 2015). Indeed numerous investigations based on assessments of chlorophyll a fluorescence have shown that PSII was quite resistant to water deficits, being either unaffected (Lawlor and Cornic, 2002).

If positive correlation has been observed between the progress of senescence and the decrease of the Fv/Fm ratio (Schofield et al., 2005; Toivonen and DeEll, 2001), the present study showed also that Fv/Fm didn’t change with time when samples are stored under darkness or medium light exposure. It can thus be concluded that Fv/Fm and Pi are
not good indicators of the integrity of the chloroplast because they are not correlated with the photosynthetic capacity.

5. Conclusion

Light exposure during post-harvest significantly decreased the cut-edge browning of fresh-cut lettuces, which is one of the main important parameters in consumer preference. However, the way to apply light can modify the product physiology, attractiveness and dehydration. This study underlined the potential of using intermittent moderate level light (50 μmol m⁻² s⁻¹) as a short post-harvest treatment to maintain the quality of fresh-cut products. Moreover, a residual effect has been highlighted since the positive effect of light can be maintained even when products are stored back in darkness. Although there is a strong need to make further research on this topic, the present study brings some reflections according to the modalities of light application.

References


Toivonen, P.M.A., DeEll, J.R., 2001. Chlorophyll fluorescence measurements to evaluate product physiology, attractiveness and dehydration. This study underlined the potential of using intermittent moderate level light (50 μmol m⁻² s⁻¹) as a short post-harvest treatment to maintain the quality of fresh-cut products. Moreover, a residual effect has been highlighted since the positive effect of light can be maintained even when products are stored back in darkness. Although there is a strong need to make further research on this topic, the present study brings some reflections according to the modalities of light application.