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Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light

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ABSTRACT

Fresh-cut romaine lettuce (FRL) is susceptible to tissue browning and quality deterioration, and thus has short shelf-life. The effect of continuous high intensity light (HIL, 2500 lx), low intensity light (LIL, 500 lx), and darkness on FRL browning and quality was studied upon 7 d cold storage. Changes in browning index (BI), browning-related enzyme activity, quinone, total phenol (TP), ascorbic acid (AA) content, antioxidant capacity (AC), and fresh weight loss were investigated. HIL significantly decreased BI and inhibited polyphenol oxidase (PPO) and peroxidase (POD) activities, and quinone accumulation. While HIL preserved more TP and AA content and resulted in higher AC value compared to darkness. Conversely, LIL induced PPO and POD activities as well as quinone generation, resulting in higher BI compared with darkness. Meanwhile, LIL induced low both TP content and AC value that contributed to low quality property. Both HIL and LIL notably increased PAL activity and fresh weight loss that progressively increased over time compared to darkness. Conclusively, HIL exposure effectively protected FRL from browning and quality decay by inhibiting browning-related enzyme activity and maintaining nutritional constituents during refrigeration.

Industrial relevance: Romaine lettuce is consumers' favorite leafy vegetable for its crispness, good aroma, tender appearance as well as high phytochemicals like phenolic compounds. However, by nature, romaine lettuce is very perishable and susceptible to quality decay and enzymatic browning. In current retail marketing, fresh-cut produce is unavoidably exposed to light conditions during its displayed shelf-life for consumers' choice. This study investigated the effect of continuous high intensity light, low intensity light exposure and darkness on tissue browning and quality property of fresh-cut romaine lettuce. Result indicated that the high intensity light exposure was effective in inhibiting tissue browning and maintaining quality of fresh-cut romaine lettuce upon cold storage. The findings are innovative and very helpful for fresh-cut lettuce producers, distributors, and sellers to decrease the occurrence of undesirable color and nutrition changes by modifying light illumination during storage.

1. Introduction

Tissue browning in leafy vegetables is a common and serious disorder, and easily detected and rejected by consumers. From this point of view, lettuce is one of the most studied species due to its great susceptibility to tissue browning (Degl'Innocenti, Pardossi, Tognoni, & Guidi, 2007; Saltveit, 2000). Romaine lettuce is consumers' favorite leafy vegetable for its crispness, good aroma, tender appearance as well as high phytochemicals like phenolic compounds (Llorach, Martínez-Sánchez, Tomasbarberan, Gil, & Ferrers, 2008; Martínez-Sánchez, Tudela, Luna, Allende, & Gil, 2011). However, by nature, romaine lettuce is very perishable and susceptible to enzymatic browning. Unfortunately, this browning in particular midrib browning occurs faster and is more difficult to control than in other types of lettuce such as iceberg (Martínez-Sánchez et al., 2011). Various approaches have been extensively carried out to control fresh-cut lettuce browning, e.g., chemical treatment (Altunkaya & Gökmen, 2009; Chen, Zhu, Zhang, Niu, & Du, 2010; Saltveit, 2004), physical treatment (Rico et al., 2008; Zhang, Lu, Lu, & Bie, 2006) or combination of them (Roura, Pereyra, & Vallea, 2008), as well as some novel biological treatments, like green tea extracts and whey protein (Altunkaya, 2011; Martín-Diana, Rico, & Barry-Ryan, 2008). However, some of these methods are generally constrained by their toxicity, high cost, and potentially impairing organoleptic properties and nutrient content of the produce (Alothman, Bhat, & Karim, 2009; Paull & Chen, 2000). Thus the industry still needs cheap, safe, and environmental-friendly strategy to prevent browning and quality deterioration of fresh-cut produce.

Light exposure, as a non-toxic, cheap, free of residual, and environmental-friendly treatment, currently represents a novel approach to non-thermally inactivate undesirable enzymes and preserves the overall quality of fresh-cut produce in comparison with traditional

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methods that mainly base on chemical inhibitors or heat treatment (Manzocco, Quarta, & Dri, 2009). Recently, the effect of light exposure at various intensities and photoperiods on quality and physiology of fresh fruits and vegetables was extensively studied (Lester, Makus, & Hodges, 2010; Manzocco et al., 2009; Martínez-Sánchez et al., 2011; Noichinda, Bodhipadma, Mahamontri, Narongruk, & Ketsa, 2007; Zhan, Fontana, Tibaldi, & Nicola, 2009). Some of these researches concluded that high dose (12.7 W/m^2, approx. 4704 lx) of light irradiation inactivated PPO activity in model system and apple derivatives (Manzocco et al., 2009). Higher intensity light exposure was associated to nonreversible structural changes of PPO, which resulted in enzyme inactivation (Manzocco et al., 2009). The same authors speculated that PPO enzyme inactivation by light was probably proceeded through direct photo-oxidation of absorbing residues of PPO amino acids to form radicals. A 21.8 μmol/m^2/s (approx. 1817 lx) dose of light exposure prevented Chinese kale nutritional quality loss associated with ascorbic acid, glucose, and fructose (Noichinda et al., 2007). In contrast, low dose of light exposure at 6 μmol/m^2/s (approx. 500 lx) promoted fresh-cut romaine lettuce (FRL) browning (Martínez-Sánchez et al., 2011), and 4.28 μmol/m^2/s (approx. 357 lx) dose of light exposure was not beneficial to minimally processed garden cress during storage, respectively (Zhan et al., 2009).

Taking into account few literatures concerning the influence of different intensity light exposure on tissue browning and nutritional quality of fresh-cut FRL (Manzocco et al., 2009), the objective of this study is to investigate the influence of continuous high intensity light (HIL) exposure, continuous low intensity light (LIL) exposure, and darkness on FRL tissue browning and quality characteristics upon 7 d cold storage. Parameters related to tissue browning and nutritional quality, such as browning index (BI), phenylalanine ammonia lyase (PAL, EC 4.3.1.5), polyphenol oxidase (PPO, EC 1.14.18.1), and peroxidase (POD, EC 1.11.1.7) activities, total phenols (TP) and quinone content, ascorbic acid (AA), dehydroascorbic acid (DHA), antioxidant capacity (AC), as well as fresh weight loss, were assayed at pre-storing (0), 1, 3, 5, and 7 d after storage, respectively.

2. Materials and methods

2.1. Sample preparation

Romaine lettuces (*Lactuca sativa* L.) were obtained from local farm and brought to laboratory within 2 h for experiment. The plants were selected for uniformity of color and size. The selected lettuces were roughly washed with cold tap water (5 °C ± 2) for getting rid of soil. Then the outer leaves and core of washed lettuce were removed and the remaining leaves were cut into pieces (approx. 3 × 3 cm) using sharp stainless steel knife. Subsequently, all the cut leaves were washed in cold tap water (5 °C ± 2) again before surface-sterilized by immersion in 0.2% (v/v) NaClO solution (15 L/kg, pH 6.5 adjusted with citric acid) for 30 s. The excess surface water on leaves was removed by manual salad spinner (Gelin plastic Co. Ltd, Taizhou, China) for 30 s. After this, 150 g material for each sample was heat-sealed in 25 × 30 cm polypropylene film (Danisco, Bristol, UK) with O_2 permeability of 15,000 cm^3/m^2/d at 25 °C. Finally all the 45 samples were packaged according to experimental design.

The packaged samples were randomly separated into three bunches and stored at 4 °C for 7 d under three light conditions. The intensity of light on package surface for each treatment was 2500 ± 2 lx (HIL), 500 ± 2 lx (LIL), and 0.2 lx (darkness) measured with illuminometer (TES-1330A, TES electrical electronic corp. Taibei, Taiwan), respectively. The light illumination was obtained from fluorescent lights (Foshan Electrical and Light Co. Ltd., Foshan, China) equipped in light room. The different light intensities were obtained by changing the number of fluorescent lights and the distance between samples and fluorescent lights. Parameters were analyzed at pre-storing (0), 1, 3, 5, 7 d after storage.

2.2. PAL, PPO, and POD activities and protein content assay

PAL, PPO, and POD activities were measured as described by Zhan et al. (2009). For PAL, in brief, 5 g of fresh tissue of each sample was homogenized in 40 ml 50 mM sodium phosphate buffer (PBS, pH 8.0). The homogenate was centrifuged at 20,000 × *g* at 4 °C for 20 min. The resulting supernatant was collected as enzyme extract. PAL activity was carried out by mixing a 0.1 ml extract, 0.5 ml 50 mM L-phenylalanine and a 1.40 ml 50 mM PBS. After incubation at 40 °C for 1 h, the absorbance was measured at 290 nm wavelength. The enzyme activity was expressed as μmol cinnamic acid per hour per gram of fresh weight (μmol cinnamic acid/gFW/h).

PPO and POD enzymes were extracted by homogenizing 5 g fresh tissue from each sample in 40 ml of 50 mM PBS (pH 7.0). The homogenate was centrifuged at 20,000 × *g* at 4 °C for 20 min and the resultant supernatant was used as the crude enzyme source. PPO activity was determined by incubating 0.1 ml enzyme extract in 1.9 ml PBS (pH 7.0) containing 2.5 mM of pyrocatechol at 25 °C for 30 min before absorbance was recorded at 480 nm wavelength. One unit of PPO activity was defined as the amount of enzyme that causes a change of 0.01 in absorbance per minute. POD activity was measured as an increase in absorbance at 470 nm wavelength due to guaiacol oxidation. The reaction mixture contained 25 mM PBS (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and enzyme. The soluble protein content of enzyme extracts was assayed according to Bradford (1976) method.

2.3. TP content assay

The total phenolic content was measured using the Folin–Ciocalteu procedure (Singleton & Rossi, 1965). For each sample, 5 g of fresh sample was extracted in 20 ml methanol and the extract was centrifuged at 15,000 × *g* at 4 °C for 20 min. The assay involved mixture of 100 μl aliquot of the methanol extract with 500 μl of Folin–Ciocalteu reagent (Sigma-Aldrich Inc, St Louis, MO, USA). After standing for 3 min, 400 μl of 7.5% sodium carbonate/water (w/v) was added and the contents of the tubes were thoroughly mixed before incubation at 20 °C for 30 min. The absorbance at 760 nm wavelength was read and the result was expressed as milligram gallic acid (Sigma-Aldrich Inc, St Louis, MO, USA) per gram of fresh weight.

2.4. Soluble quinone content assay

The soluble quinone content was performed as description of Degl'Innocenti et al. (2007). A 5 g of tissue was homogenized with 20 ml of methanol. The homogenate was centrifuged at 15,000 *g* for 15 min. The supernatant was used to directly measure the soluble quinone at 437 nm wavelength. And the result was expressed as the absorbance of per gram fresh material.

2.5. BI assay

BI was estimated as described by Pen and Jiang (2003). For each replicate, 10 pieces of cut leaves were scaled according to their browning area based on the following browning scale standard: 0 = no browning, 1 = browning spots, 2 = slight browning (<1/5), 3 = moderate browning (1/5–1/4), 4 = moderate-serious browning (1/4–1/2), 5 = serious browning (>1/2). The BI was calculated as the following formula: BI = ∑ (browning scale × percentage of corresponding of leaves within each scale). Sample with BI more than 2.0 was considered unmarketable.

2.6. AA and DHA content analysis

AA and DHA were determined spectrophotometrically according to protocol described by Kampfenkel, Montagu, and Inzé (1995) and Zhan et al. (2009). The DHA content was computed from the difference between the total AA and AA. The results were expressed as