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Light exposure during storage preserving soluble sugar and L-ascorbic acid content of minimally processed romaine lettuce (*Lactuca sativa* L.var. *longifolia*)

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ABSTRACT

Minimally processed romaine lettuce (MPRL) leaves were stored in light condition (2500 lux) or darkness at 4 °C for 7 d. Light exposure significantly delayed the degradation of chlorophyll and decrease of glucose, reducing sugar, and sucrose content, and thus preserved more total soluble solid (TSS) content at the end of storage in comparison with darkness. While, it did not influenced starch content that progressively decreased over time. The L-ascorbic acid (AA) accumulated in light-stored leaves, but deteriorated in dark-stored leaves during storage. The dehydroascorbic acid (DHA) increased in all leaves stored in both light and dark condition, of which light condition resulted in less DHA than darkness. In addition, the fresh weight loss and dry matter significantly increased and these increases were accelerated by light exposure. Conclusively, light exposure in applied intensity effectively alleviated MPRL quality deterioration by delaying the decreases of pigments, soluble sugar, TSS content and accumulating AA.

1. Introduction

Romaine lettuce is consumers' favourite leafy vegetable for its crispness, good aroma, tender appearance and high phytochemicals like phenolic compounds (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008; Martínez-Sánchez, Tudela, Luna, Allende, & Gil, 2011). Unfortunately, romaine lettuce is naturally perishable and susceptible to quality deterioration after harvest and thus has a short shelf-life. Various chemical treatments such as reducing agents and browning inhibitors have been applied to control tissue browning in order to alleviate quality loss (Roura, Pereyra, & Vallea, 2008; Saltveit, Choi, & Tomás-Barberán, 2005). However, consumers' concerns about chemical toxicity preclude the chemical application. Therefore, it is urgent to seek alternatives to preserve fresh romaine lettuce quality without use of chemicals to meet consumer acceptability.

Light exposure currently represents a novel approach and is widely used to preserve the overall quality of fresh produce (Lester, Makus, & Hodges, 2010; Manzocco, Quarta, & Dri, 2009; Noichinda, Bodhipadma, Mahamontri, Narongruk, & Ketsa, 2007; Toledo, Ueda, Imahori, & Ayaki, 2003; Zhan, Hu, Li, & Pang, 2012a; Zhan, Li, Hu, Pang, & Fan, 2012b). In comparison with traditional preservative methods, light exposure is a non-toxic, cheap, free of residuals, and environmental-friendly treatment (Manzocco et al., 2009). In view of this, extensive studies were carried out to investigate the effect of light exposure at various intensities and photoperiod on quality and physiology of fresh fruits and vegetables during postharvest storage (Büchert, Gómez Lobato, Villarreal, Civello, & MartIneza, 2011; Lester et al., 2010; Martínez-Sánchez et al., 2011; Noichinda et al., 2007; Olarte, Sanz, Echávarri, & Ayala, 2009; Zhan et al., 2012a, 2012b). More recently, we found that intensity of 2500 lux light exposure effectively protected fresh-cut romaine lettuce from browning and quality decay by inhibiting browning-related enzyme activity and maintaining nutritional constituents during refrigeration (Zhan et al., 2012b). Continuous white light illumination during postharvest storage supports photosynthetic capacity of fresh spinach leaves, resulting in increased availability of soluble carbohydrates (Toledo et al., 2003). Like spinach leaves, romaine lettuce leaves are green and tender when harvested and can continuously photosynthesize by which the light energy is converted to chemical energy and stored in the bonds of sugar. Thus, we hypothesise that light exposure during postharvest storage may regulate carbohydrate metabolism of romaine lettuce leaves.

The aim of this study is to determine how continuous light exposure (2500 lux) affects minimally processed romaine lettuce (MPRL) carbohydrate content associated with glucose, reducing sugar, sucrose, starch, and total soluble solid (TSS) content upon 7 d cold storage. In addition, the chlorophyll, L-ascorbic acid (AA), dehydroascorbic acid (DHA), dry matter content, and fresh weight loss were also evaluated during storage, respectively.

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2. Materials and methods

2.1. Sample preparation

Romaine lettuces (*Lactuca sativa* L.var. *longifolia*) were obtained from local farm and brought to laboratory within 2 h for experiment. The plants were selected for uniformity of colour and size. The selected lettuces were washed with cold tap water (5 °C ± 2) for getting rid of soil. Then, the roots, outer old leaves, and core of washed lettuce were removed using sharp stainless steel knife. The remaining leaves were subsequently 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 air-dried at 12 °C for 20 min. After this, about 200 g material for each sample was arranged one layer in 35 cm × 30 cm plastic tray (Botong plastic Co. Ltd, Beijing, China) and wrapped with 35 μm polypropylene film (Tianjin Luda Ltd. Corp., Tianjin, China) with O2 permeability of 6,800 cm^3/m^2 d at 25 °C to minimise desiccation.

All of 18 packaged samples were randomly separated into two bunches. One bunch with nine samples were stored under light (2500 ± 2 lux) condition and the other bunch with nine samples were stored under dark (0.2 lux) conditions at 4 °C for 7 d, respectively. The light illumination was obtained as described in our previous report (Zhan et al., 2012b). Parameters were analyzed at pre-storing (0), 1, and 7 d after storage.

2.2. Pigment content analysis

A 10 g fresh tissue from each sample was blended and 2 g blending paste was extracted in 20 ml 80% acetone/water (v/v) overnight at 4 °C in dark condition. The extract was centrifuged at 3000*g* for 10 min and supernatant was used for the spectrophotometric determination of chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) at wavelength of 662 and 645 nm, respectively. For each treatment and each period, three samples were used. The amount of these pigments was calculated according to the Lichtentaler and Wellburn method (1983).

2.3. Soluble sugars, starch and TSS content measurement

About 100 g fresh leaves from each sample were dried in an oven (DHG-9053A, Shanghai Heheng Instrument & Equipment Co. Ltd., Shanghai, China) at 65 ± 1 °C until constant weight. The dried leaves were transferred to mortar and ground into powder using as measurement of sugar and starch. For extraction of soluble sugars and starch, accurate 0.050 g dried power was extracted in 10 ml 85% ethanol solution and incubated in water bath at 60 °C for 20 min. Then, the extract solution was centrifuged at 3000*g* for 10 min and the supernatant was collected. The pellet was re-extracted twice with same solvent and the supernatants were combined. The total supernatant was filtered through Whatman filter paper (Grade 1, Hangzhou Whatman-Xinhua filter paper Co., Ltd., Hangzhou, China) after adding activated carbon in order to remove pigments. The filtrate was collected for soluble sugar measurement. The pellet left after extraction of soluble sugars was extracted in 5 ml distiled water and 6.5 ml 52% perchloric acid for determination of starch as proposed by Morris (1948).

Glucose, sucrose, and starch were estimated by anthrone-sulphuric acid method of Yem and Willis (1954) and Morris (1948). Standard curve was plotted with glucose and sucrose, respectively. Reducing sugars were quantified by the 3, 5-dinitrosalicylic acid method at 540 nm wavelength as proposed by Miller (1959) with glucose as standard curve.

TSS content was measured using hand refractometer (Model N1; Atago, Tokyo, Japan). Briefly, 10 g fresh tissue from each sample was ground and the grinding paste was subsequently squeezed through four-layer cotton cloth. A few drops of squeezed juice were dropped onto refractometer window for reading Brix value. The results were expressed in degree Brix.

2.4. AA and DHA content analysis

AA and DHA were determined spectrophotometrically according to the methodology of Kampfenkel, Montagu, and Inzé (1995). A 20 g of fresh material was ground in 40 ml 6% of freezing trichloroacetic acid/water (w/v) on ice. The homogenate was centrifuged at 15,000*g* at 4 °C for 10 min and the resultant supernatant was immediately used for total AA and AA analysis. The DHA content was computed from the difference between the total AA and AA. The results were expressed as milligram per 100 grams of fresh weight (mg/100 g FW) based on the calibrations compared with standard curves produced by freshly prepared L-ascorbic acid and dehydroascorbic acid.

2.5. Fresh weight loss and dry matter content assay

Both fresh weight loss and dry matter content assay were measured according to our previous methodology (Zhan et al., 2012a). The results were expressed as percentage.

2.6. Statistical analysis

All data in triplicates from three samples for each treatment were submitted to the analysis of variance using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as the means ± SD. One-way ANOVA was applied to compare the effect of light condition on measured parameters using the least significant difference (LSD) test at 0.05 confidence level.

All the extractions were carried out under the lab light condition (approx. 200 lux) with the exception of extra explanation and all the chemicals were analytical reagents purchased from Huafeng chemical reagent Co. Ltd. (Zhengzhou, China) and Sangon Biotech Co. Ltd. (Shanghai, China). And all the spectrophotometric analyses were conducted using Shimadzu spectrophotometer (UV-2401PC, Shimadzu Co. Ltd, Kyoto, Japan).

3. Results and discussion

3.1. Chlorophyll content

Light exposure significantly affected MPRL pigment content that displayed progressive degradation over time regardless of treatments (Fig. 1). Chl *a* content showed significant decrease in all leaves during storage (Fig. 1A). However, compared to dark-stored leaves, light stored leaves preserved remarkably more Chl *a* during storage, implying that light condition was efficient to alleviate Chl *a* degradation. Similar to Chl *a*, Chl *b* content in samples stored under both light and dark condition deteriorated over time, and this deterioration was markedly delayed by light exposure (Fig. 1B). At first and seventh day storage, Chl *b* content in samples exposed to light condition were more 20% and 22% than that in samples stored in darkness, respectively. The total chlorophyll content, sum of Chl *a* and Chl *b*, showed consistent tendency with Chl *a* during storage (Fig. 1C).

Yellowing of green leafy vegetables upon storage is normally considered to be the major consequences of chlorophyll degradation (Brown, Houghton, & Hendry, 1991). In the present study, MPRL leaves de-greened progressively with the chlorophyll