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Effect of blue light treatment on fruit quality, antioxidant enzymes and radical-scavenging activity in strawberry fruit

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ABSTRACT

The effect of blue light treatment on quality, antioxidant capacity and enzyme activity of strawberry fruit stored at 5 °C was evaluated. The results showed that blue light illumination increased the color index, respiration rate and ethylene production in strawberries during storage. The treatment also enhanced the activities of antioxidant enzymes including superoxide dismutase, catalase and ascorbate peroxidase, and maintained lower levels of superoxide anion, hydrogen peroxide, and malondialdehyde. In addition, the 1,1-diphenyl-2-picrylhydrazy (DPPH) radical-scavenging activity, total phenolic, ascorbic acid, total sugar content, as well as titratable acidity (TA) were increased by blue light exposure. These data suggested that blue light treatment might maintain quality and improve nutritional value of strawberry fruit due to the enhancement of their antioxidant systems and free radical-scavenging capabilities.

1. Introduction

Strawberry fruit is widely consumed, both as fresh and process products. It is rich in natural antioxidants, including anthocyanin, flavonoids and phenolic compounds (Erkan et al., 2008). High consumption of strawberries has been associated with a lowered incidence of chronic diseases (Zhang et al., 2008). Due to health awareness, it is of great interest to enhance antioxidant capacity during postharvest storage of strawberries.

Among various environmental factors, light is one of the most important variables affecting the phytochemical concentrations in plant (Samuolienė et al., 2012). Red and blue light have the greatest impact on plant growth and biosynthesis of secondary metabolites because they are the major energy sources for photosynthetic CO_2 assimilation in plants (Lin et al., 2013). For example, exposure to red light has been shown to increase lycopene accumulation in tomato (Liu et al., 2009). Blue light-emitting diodes (LEDs) could regulate the metabolic pathways in red leaf lettuce, which resulted in increased concentrations of health-promote compounds and plant growth (Stutte et al., 2009). Supplementation of blue light also improved the antioxidant activity of *Kalanchoe pinnata* and changed the phenolic profile of the extracts (Nascimento et al., 2013).

It has been reported that the levels of bioactive compounds and antioxidant capacity in strawberries could be enhanced by postharvest treatments, such as benzo-thiadiazole-7-carbothioic acid *S*-methylester (BTH), UV-C radiation, abscisic acid (ABA) (Cao et al., 2011; Erkan et al., 2008; Li et al., 2014). However, literature concerning the effect of blue light illumination on strawberry fruit during postharvest storage is not abundant and little is known about what happens after blue light treatment. The purpose of this study was to explore not only the changes in the antioxidant capacity but also the physiological quality in strawberry fruit exposed to blue light.

2. Materials and methods

2.1. Plant materials

Strawberry (*Fragaria ananassa* Duch. cv. Fengguang) fruit were harvested by hand at ripe stage 7 from a farm at Ningbo, Zhejiang province and transported within 2 h to the laboratory. All fruit were selected for uniform size and color, and then divided randomly into two groups.

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2.2. Treatment and storage

For blue light treatment, the first group of fruit were irradiated with blue (470 nm) light at an intensity of 40 μmol m^{-2} s^{-1} for 12 days at 5 °C (80–90% relative humidity). The second group of fruit, which was non-illuminated blue light, was considered as the control and stored at 5 °C in the dark. Control and illuminated fruit were taken initially and at 2-day intervals during storage and immediately frozen in liquid nitrogen and the samples were stored at −80 °C until analysis.

2.3. Color index assessment

Superficial color was measured for all strawberries samples, employing the parameter of CIRG = (180 − *h*)/(*C* + *L*) (Carreño et al., 1995) with a Minolta CR-410 colorimeter, using the CIELAB color system. *L* is the lightness and corresponds to a black–white scale, *h* is the hue angle on the color wheel, and *C* is the chroma, a measure of the intensity of color.

2.4. Respiration rate and ethylene production determination

Three replicates of five fruit each were held in gas-tight jars at 5 °C for 2 h prior to gas sampling. CO_2 was measured with an infrared gas analyzer (GXH-305, Beijing, China), and results of respiration rate were expressed as mg kg^{-1} h^{-1}. Ethylene production was monitored in a Shimadzu 14-A gas chromatograph with flame ionization detector, and the results were expressed as μl kg^{-1} h^{-1}.

2.5. Contents of ascorbic acid, titratable acidity (TA), and total sugar

One gram of frozen tissue was grinded and extracted with 5 ml 5% trichloroacetic acid (TCA), then centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was used to ascorbic acid determination. Ascorbic acid content assay was carried out according to Kampfenkel et al. (1995) method.

TA content was determined by titrating 5 ml juice with 0.1 mol/L NaOH to pH 8.2 and was expressed as % citric acid.

One gram of flesh tissue was ground with 5 ml ethanol and the mixture was centrifuged at 12,000 × *g* for 10 min at 4 °C. After that, 0.1 ml of ethanol extract was mixed with 1 ml of 2 g/L anthrone in 706 g/L H_2SO_4. The mixture was incubated at 100 °C for 15 min, cooled in a water bath and the total sugar content was measured absorbance at 625 nm.

2.6. Total phenolic content and DPPH radical scavenging activity

To prepare the fruit extract, 5 g samples from each replica were homogenized with 5 mL of precooled 95% ethanol containing 3% formic acid (v/v), and after centrifugation at 10,000 × *g* for 15 min (4 °C), another 15 mL of 80% ethanol containing 5% formic acid (v/v) was used to extract the residue again. The supernatant was combined to make the final volume of 25 mL for analysis. Total phenolics content in strawberry fruit extracts was determined according to the Folin-Ciocalteu procedure (Slinkard and Singleton, 1977). The results were expressed as milligrams of gallic acid per gram of tissue.

The DPPH radical-scavenging activity of strawberry fruit was estimated following the method of Larrauri et al. (1998) with slight modifications. The above ethanol extract (0.1 ml) was added to 2.9 ml of DPPH (120 μM) in methanol and absorbance was measured at 517 nm after the reaction mixtures were incubated for 30 min at 30 °C in the dark. The result was calculated according to the following formula: DPPH radical scavenging activity (%) = 1 − (absorbance of sample/absorbance of control) × 100%. Vitamin C was used as a standard antioxidant analyzed at the same time. The final results were calculated and expressed as vitamin C equivalents (VCE) per gram on a fresh weight basis.

2.7. Antioxidant enzyme assays and measurements of malondialdehyde (MDA), superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2)

For superoxide dismutase (SOD, EC 1.15.1.1) and O_2^- production, 1 g of flesh tissue was ground with 5 ml of 50 mM sodium phosphate buffer (pH 7.8). For catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11), fruit tissue (1 g) was homogenized with homogenized with 5 ml of 50 mM sodium phosphate buffer (pH 7.0). One gram sample was extracted with 5 ml of 100% acetone and then centrifuged at 12,000 × *g* for 10 min at 4 °C for H_2O_2 content.

The activities of SOD and CAT were assayed as described by Toivonen and Sweeney (1998). Reaction medium contained 0.05 M phosphate buffer (pH 7.8), 13 mM methionine, 3 μM EDTA, 63 μM nitro blue tetrazolium (NBT), 1.3 μM riboflavin and 0.1 ml of crude enzyme extract. One unit of SOD was the amount of extract that gives 50% inhibition of the reduction rate of NBT. CAT activity was determined by monitoring the enzyme-catalyzed decomposition of H_2O_2 by potassium permanganate. The reaction mixture consisted of 20 μl enzyme extract, 50 mM sodium phosphate buffer (pH 7.0), 12.5 mM H_2O_2.

APX activity measurement was adapted from the method of Nakano and Asada (1981). Reaction medium was contained 2.8 ml of 50 mM sodium phosphate buffer (pH 7.0), 0.1 ml of enzyme extract, 10 μl of 30% H_2O_2, and 0.1 ml of 9 mM AsA. One unit of APX enzyme activity was defined as the amount of enzyme that produced an OD_{290} reduction per minute under the assay conditions.

MDA content was measured according to Yang et al. (2008) with some slight modifications. The supernatant (2 ml) was precipitated with 2 ml 0.67% (w/v) 2-thiobarbituric acid. The reaction mixture was incubated in a water-bath shaker at 100 °C for 30 min. The amount of MDA was estimated as millimolar per gram FW.

O_2^- production was determined using the method of Elstner and Heupel (1976). O^{2-} production was calculated against the standard curve and expressed as nmol g^{-1}FW min^{-1}.

Protein content in the enzyme extracts was determined according to Bradford (1976) method, using bovine serum albumin as a standard.

2.8. Statistical analysis

Experiments were performed using a completely randomized design. Statistical analysis was performed using the SPSS package program version 16.0 (SPSS Inc., Chicago, IL). All data were expressed as the mean ± standard error (SE) and analysis by one-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effect of blue light treatment on color index in strawberry fruit

CIRG value was used to express as fruit color of strawberry fruit in our study. No significant change in CIRG value was observed in control strawberries during storage. However, the value in the blue light-treated samples tended to increase gradually, which exhibited a higher level than that in the control after 4 days of storage (Fig. 1).