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RESEARCH ARTICLE

Effect of Light on Anthocyanin Levels in Submerged, Harvested Cranberry Fruit

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Anthocyanins are a group of plant antioxidants known for their therapeutic use. The effects of natural light, red light, and far-red light on individual as well as total anthocyanin content in cranberry fruit (*Vaccinium macrocarpon* Ait) were examined in an experimental setting designed to mimic water-harvesting conditions. The reversed-phase high-performance liquid chromatography (HPLC) method was used to separate and analyze the anthocyanins. In contrast to the case of the control sample that was kept in the dark, natural light increased the total anthocyanin level by 75.3% and 87.2% after 24 and 48 hours of water immersion, respectively. Red light and far-red light increased the total anthocyanin level by 41.5% and 34.7%, respectively. The amount of each individual anthocyanin increased differently under natural light, red light, and far-red light, suggesting that expressions of enzymes that catalyze the anthocyanin biosynthesis are regulated differently by environments.

INTRODUCTION

The quality and commercial value of American cranberry fruit (*Vaccinium macrocarpon* Ait) are determined by their color [1]. The red color of cranberry fruit is due to the presence of anthocyanins. Anthocyanins have important therapeutic values, including antitumor [2, 3], antiulcer [4], antioxidant, and anti-inflammatory activities [5]. Six anthocyanins have been reported in cranberries based on the high-performance liquid chromatography (HPLC) analysis of acid-alcohol fruit extracts on reversed-phase C_{18} column. These are cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, peonidin 3-galactoside, peonidin 3-glucoside, and peonidin 3-arabinoside [6, 7]. The proportions of individual anthocyanins in cranberry fruit may affect the color stability of cranberry products such as juice and sauce [8, 9]. Yan et al [10] reported that cyanidin 3-galactoside showed antioxidant activity superior to six other monoglycosides of quercetin and myricetin isolated from cranberry fruit as well as vitamin E by evaluating compounds for 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity and ability to inhibit low-density lipoprotein oxidation in vitro.

Light has been shown to be the most important environmental factor influencing anthocyanin biosynthesis in plants [11], although in some species, such as *Vitis vinifera* cv. Shiraz anthocyanin accumulation appears not to be light-sensitive [12]. Phytochromes are among the most extensively researched photoreceptors which sense light, and are known to be involved in anthocyanin biosynthesis [13, 14, 15]. Phytochromes respond to red (660 nm) and far-red (730 nm) light, and direct plant gene expression by switching between the red-absorbing form (Pr) and the far-red absorbing form (Pfr). Previously, we have examined the effect of various wavelengths of light on the development of the cranberry plant and anthocyanin biosynthesis in cranberries which were still attached to the plant. We have observed that red light stimulates flowering and anthocyanin biosynthesis in cranberry plant and fruit, respectively [16].

In general, leaves and stems decrease light exposure for berries lower on the plant. Preharvest anthocyanin content of cranberries at the bottom and the top of the plant varies significantly, primarily due to the differences in light accessibility [17].

Water-harvesting has become a common practice in the cranberry industry, and it is accomplished by flooding the cranberry bog with water to float the buoyant fruit for easy collection. However, potential effects on the berries due to the water-harvest technique have not been studied systematically. One study did show that prolonged fruit immersion increases fungal rot of the berries [18]. During the water-harvest, cranberries on the surface of the water receive the same or more light than the fruit still attached to the plant. In this paper, we evaluate the effects

of natural light, red light, and far-red light on individual as well as total anthocyanin levels in cranberry fruit under conditions that mimic water-harvesting.

MATERIALS AND METHODS

Plants

Cranberries (*Vaccinium macrocarpon* Ait, cv "Early Black") used in this study were obtained from the bog of the Cranberry Experiment Station, the University of Massachusetts, East Wareham, Mass, in October 1999.

Light sources

Red light, with a photon fluence rate of 12 μmole $m^{-2}s^{-1}$, was obtained from six 40-w fluorescent tubes (F48T12/R-660/HO, Red, General Electric Company, USA) filtered through a red plastic sheet (Roscolux color filter # 27, ROSCO Laboratories, Port Chester, NY). Far-red light, with a photon fluence rate of 5 μmole $m^{-2}s^{-1}$, was obtained from 500-w brilliant white light halogen double-ended quartz FCL bulbs (Osram Sylvania Products Inc, Winchester, Ky) filtered through 3 mm far-red plastic (type FRF700, West Lakes Plastics, Lenni, Pa). Light sources in each case were kept at a distance of 0.8 meter from the berries. All light measurements were made with a Model IL1400A Radiometer/Photometer (International Light, Inc, Newburyport, Mass).

Experimental setting

Cranberries were taken from a flooded bog after the harvest machine had knocked the fruit loose from the vines and selected in approximate same size and color for experiment in order to avoid variability in the anthocyanin content. The fruit were randomly divided into five groups and held in beakers containing water. Two sizes of beakers (1000 mL and 250 mL) were used. The 1000 mL beaker (diameter: 11.6 cm) contained 800 mL of water, and approximate 34 berries forming just one layer on the surface of the water were placed in the beaker. The 250 mL beaker (diameter: 7.5 cm) contained 200 mL of water, and approximate 17 berries forming just one layer on the surface of the water were placed in the beaker. Two groups in the 1000 mL beakers were placed in a nursery area outside the laboratory and received a cycle of daylight for 24 and 48 hours, respectively. The remaining three groups in the 250 mL beakers were placed in a temperature-controlled darkroom at 20°C. One of these 250 mL beakers was kept in the darkroom and was used as a control sample. The other two, also kept in the darkroom, received 30 minutes of red light or far-red light per day, respectively, for two days. The berries from the two groups placed outside were collected after 24 and 48 hours, respectively, and the fruit from the three groups placed in the temperature-monitored room were individually collected after 48 hours. Eight grams of the berries from each group were weighed and homogenized in 10 mL of ethanol: 1.5 N HCl (85 : 15, v/v) to extract the anthocyanins overnight at 4°C. The sample extracts were filtered through 0.2 μm filters before injection into the HPLC column.

Table 1. Effect of light on total anthocyanin level in submerged, harvested cranberry fruit.

Light treatment	Total anthocyanin (mg)/100 g fresh fruit
Natural light (48 h)[a]	35.47* ± 2.39
Natural light (24 h)[b]	33.24* ± 1.47
Dark	18.95 ± 0.88
Red light	26.82* ± 1.6
Far-red light	25.53* ± 2.89

Values are expressed as mean ± SE (n = 3).

a: water immersion time was 48 hours.

b: water immersion time was 24 hours.

*$P < .02$.

HPLC analyses

HPLC analyses of anthocyanins were carried out on a Waters 515 Dual Pump HPLC system, equipped with a 996-photodiode-array detector and a C_{18} column (4.6 × 150 mm) with 5 μm particle size (Waters Corporation, Milford, Mass). The software used to control the HPLC system and data analysis was Millennium 32 (Waters Corporation, Milford, Mass). Elution was carried out using a mobile phase formed by a linear gradient of (A) H_2O-acetic acid (10 : 1) and (B) methanol-acetic acid (10 : 1), with 100% (A) at 0 minute to 40% (A) and 60% (B) at 20 minutes. The flow rate was fixed at 0.2 mL/min. Anthocyanin separation and elution were detected by monitoring absorbance at 535 nm. Anthocyanin content was calculated in absolute quantities using the extinction coefficient ($\varepsilon^{1\%}_{1\,cm}$) at 535 nm as 982 [19].

RESULTS AND DISCUSSION

Composition of anthocyanins plays a role in their therapeutic effects [20]. Although six anthocyanins have been identified in cranberries [21, 22, 23], biosynthesis of those individual anthocyanins in response to environmental conditions such as light is not understood. In an attempt to elucidate anthocyanin biosynthesis, we measured total as well as individual anthocyanin content in cranberry fruit under different light conditions in an experimental setting designed to mimic water-harvesting conditions.

Statistical analysis (Student t test) was performed to detect the statistical difference between total anthocyanin content under natural light (48-hour and 24-hour), red light, and far-red light conditions and that under dark conditions. Table 1 shows that the total anthocyanin level varied significantly (> 98% confidence level ($P < .02$)) when the submerged, harvested cranberries were exposed to various light conditions. The total anthocyanin content of berries before exposure to any experimental light conditions was 18.95 ± 0.88, and was the same as the

Table 2. Percentage of anthocyanin increased in submerged, harvested cranberries exposed to different light conditions in comparison with the control, which was kept in the dark.

	Natural light (48 h)	Natural light (24 h)	Red light	Far-red light
Cyanidin 3-galactoside	89.3*	69.0*	29.1***	17.0***
Cyanidin 3-glucoside	53.8***	38.5***	71.8***	92.3***
Cyanidin 3-arabinoside	77.5*	68.2*	30.6**	30.3**
Peonidin 3-galactoside	99.6*	92.5*	43.5*	35.1*
Peonidin 3-glucoside	100.0*	80.7*	54.4*	45.6*
Peonidin 3-arabinoside	72.4*	72.4*	72.4*	69.8*
Total anthocyanins	87.2*	75.3*	41.5*	34.7*

*$P < .02$; **$.05 < P < .1$; ***$.1 < P < .5$

control that was kept in the dark. Compared to the control, cranberries exposed to one 24-hour day-night cycle had 75.3% higher anthocyanin content, and berries exposed to a 48-hour day-night cycle posted only a small further increase (87.2%). Red and far-red light had substantially less effect on total anthocyanin biosynthesis than natural light (75–87% vs. 35–42%). Red light increased total anthocyanin content (41.5%) more than far-red light (34.7%).

Separation of cranberry anthocyanins by reverse-phase HPLC revealed six anthocyanins which were assumed to be cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, peonidin 3-galactoside, peonidin 3-glucoside, and peonidin 3-arabinoside (Table 2) according to previous reports [6, 24]. The relative amounts of the six anthocyanins in the control samples which were kept in the dark (Figure 1) are consistent with earlier reports [6, 24]. Variation in different individual anthocyanins under different light conditions was also subjected to the statistical analysis (Student-test), which showed significant differences except for the cyanidin 3-glucoside under each light condition, and for cyanidin 3-galactoside under red light and far-red light conditions, as shown in Table 2. Compared with the dark conditions, the natural light conditions enhanced all the anthocyanins substantially, 72%–100% (in the 48-hour cycle), except for the cyanidin 3-glucoside, which increased by 54% (Table 2), whereas the red and far-red light had the most prominent effect on cyanidin 3-glucoside and peonidin 3-arabinoside, showing 70–92% increase (Table 2). The biosynthesis of cyanidin 3-galactoside was least affected by red and far-red light, showing only 29 and 17% increase, respectively (Table 2).

Light-dependent anthocyanin biosynthesis significantly depends on plant species and experimental conditions [13]. Although experimental conditions in our previous study [16] were different (30 minutes of light treatments per day for eight days), results had shown that red light and sunlight increased anthocyanin biosynthesis more than the far-red light did, consistently with the conclusions of the present study. However, in the above two cases—cranberry fruit that were still attached to the plant and cranberry fruit that were no longer attached to the plant, the effect of far-red illumination appeared to be close to the effect of red light, not similar to the dark control. Exposure of etiolated normal seedlings of *Brassica rapa* to red light and far-red light showed that far-red illumination enhanced more anthocyanin synthesis than red light [25]. Study of different phytochromes in Arabidopsis photomorphogenesis has shown that phytochrome A regulates plant responses to far-red light irradiation, whereas phytochrome B plays a predominant role in responses to red light irradiation [26]. Therefore, it is considered that coactions between different photoreceptors involved in the effects of red light and far-red light on anthocyanin content in cranberry fruit are as coactions between the photoreceptors involved in flavonoid biosynthesis [27].

In addition, anthocyanins contain two parts in their structures: aglycones and sugars. The biosynthesis of anthocyanins was catalyzed by several enzymes from PLA (phenylalanine ammonia-lyase), C4H (cinnamic acid 4-hydroxylase), 4CL (4-coumarate:CoA ligase), CHS (chalcone synthase), CHI (chalcone isomerase), F3H (flavanone 3 β-hydroxylase), DFR (dihydroflavonol 4-reductase), AS (anthocyanin synthase) through 3GT (UDP-glucose:flavonoid 3-O-glycosyl transferase). CHS is the first committed enzyme of flavonoid biosynthesis. AS is the first committed enzyme of anthocyanin biosynthesis. The expressions of the above enzymes are regulated differently by environments such as light and temperature. This results in the disproportional increase of different anthocyanins such as peonidin 3-glucoside compared to cyanidin 3-glucoside, due to the different aglycones although same sugar; or cyanidin 3-galactoside compared to cyanidin-3-glucoside, due to the different sugars although same aglycon.

This study demonstrates that during water-harvesting conditions, where the berries are no longer attached to the plant, exposure of the berries to light still results in increased anthocyanin levels. This study also shows that levels of individual anthocyanins increase differently following different light exposure such as natural light, red light, and far-red light. The variation in composition of anthocyanin may be manipulated to obtain a more valuable antioxidant product from cranberries. This study also contributes to our understanding of cranberry anthocyanin biosynthesis under water-harvesting conditions.

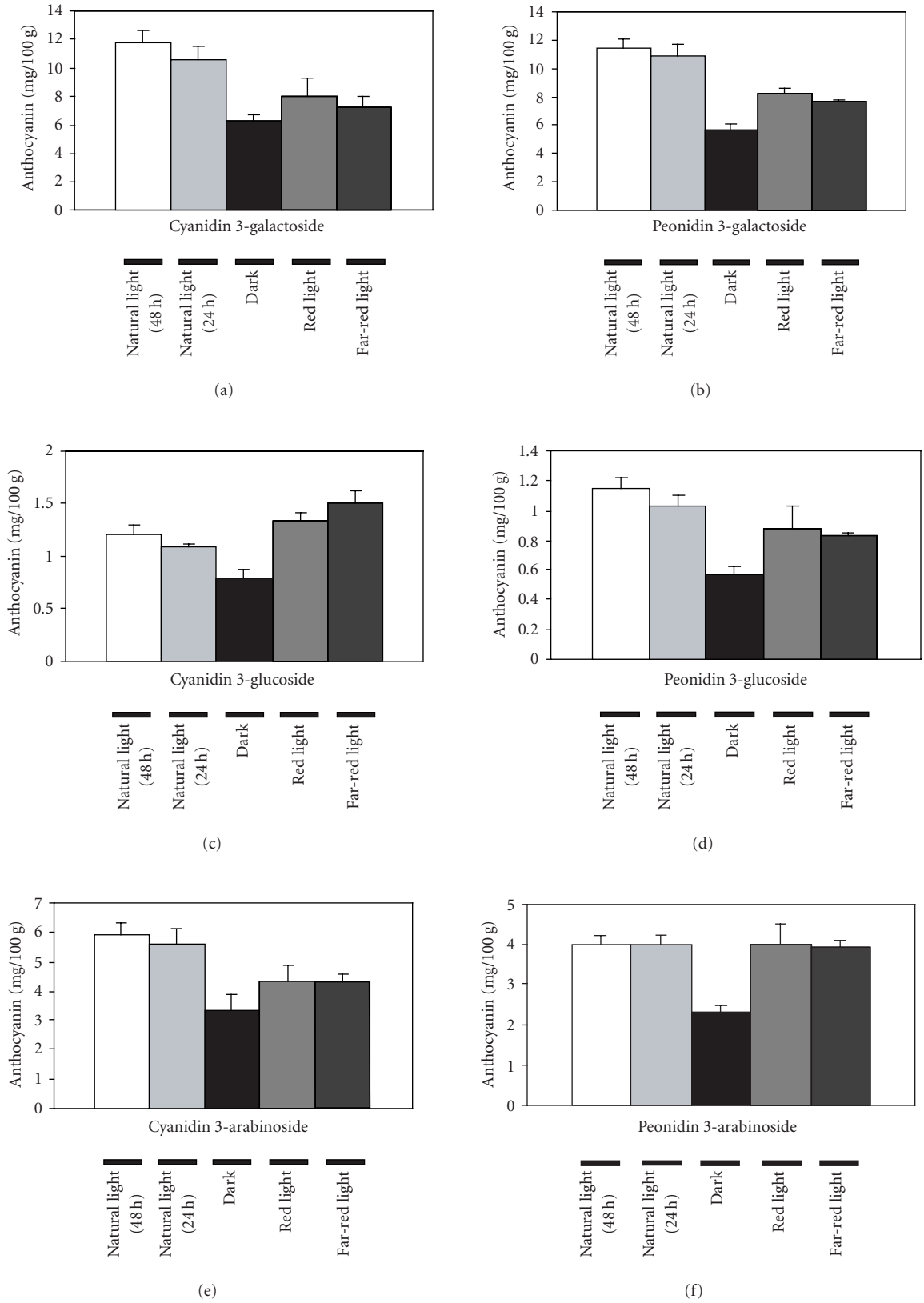

Figure 1. Effect of light on individual anthocyanin levels in submerged, harvested cranberry fruit. Cranberries were exposed to different light conditions and individual anthocyanin content was analyzed. Different light conditions, natural light (48 h), natural light (24 h), dark, red light, and far-red light, are indicated in the bottom. Values are mean from triplets with standard error bars.

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