Decrease in Nitrate Concentration in Leafy Vegetables Under a Solid-state Illuminator

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Abstract. We report on a substantial reduction of nitrate concentration in leafy vegetables that were subjected to short-term preharvest treatment by narrow-bandwidth red light of high photosynthetic photon flux density generated by a solid-state illuminator. Lettuce (*Lactuca sativa* cv. Grand Rapids), marjoram (*Majorana hortensis*, Moench.), and green onions (*Allium cepa*, L. cv. Lietuvos didieji) were grown to harvest time within a greenhouse under daylight with supplementary lighting provided by standard high-pressure sodium lamps ($130 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). A subsequent 3-day treatment within a phytotron under 638-nm light-emitting diodes ($500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) resulted in the reduction of nitrate concentration by 44% to 65%. The reduction of nitrates was accompanied by an increased concentration of nutritionally valuable carbohydrates, which is also in line with stimulation of expression of nitrate reductase by photosynthetic metabolites. Another indicator of nutritional quality, the content of vitamin C, exhibited some variation that was not directly correlated with the nitrate reduction rate that may be attributed to carbohydrate content variation and leaf tissue aging.

An important nutritional quality factor of vegetables is low nitrate content. Although data on potential long-term health risk of nitrate are contradictory (Gangolli et al., 1994; Walker, 1990), some national and international regulatory agencies are setting maximal allowed levels for nitrate in vegetables, which constitute the main dietary intake of nitrate (Santamaria, 2006). This concern is attributable mainly to the fact that $\approx 5\%$ of the ingested nitrate, which is relatively harmless, is converted to toxic nitrites and *N*-nitrosocompounds through mammalian and bacterial metabolism. Large amounts of nitrate may be present in leaves and petioles of many leafy

vegetables such as lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), celery (*Apium graveolens* L.), rocket (*Eruca sativa*, Mill.), marjoram (*Majorana hortensis*, Moench.), and green onion (*Allium cepa* L.) that have high nitrate accumulation capacity (in excess of $1000 \text{ mg}\cdot\text{kg}^{-1}$ of fresh mass). Commonly, the nitrate content in vegetables increases with increasing the geographical latitude of an agricultural region as a result of substitution of electric light sources for deficient sunlight and use of increased concentration of fertilizers in soil. Low concentration of nitrate and the presence of vitamin C, which could reduce the negative health effect of nitrate, are important factors that determine the biochemical quality of leafy vegetables (Premuzic et al., 2001). In addition, vitamin C accumulated in metabolically active tissues such as leaves acts as a signaling molecule that coordinates a protective mechanism of the oxidative system (Pastori et al., 2003).

Reduction of nitrate to nitrite in plants is a well-established biochemical process,

which is initiated by nitrate reductase (NR) (Beevers and Hagemann, 1969; Campbell, 1999; Hoff et al., 1994). Nitrate reductase is subjected to regulation by several factors. The triggering signal for the NR expression is the presence of nitrate (Crawford, 1995). Another important factor of NR regulation is irradiance, which is complex and depends on the developmental stage of the plant (Lillo, 2004; Lillo and Appenroth, 2001; Mohr et al., 1992). Light-induced regulation of NR occurs through phytochrome and metabolic products of photosynthesis. The physiologically active form of phytochrome (P_{fr}), which is activated by red light, stimulates gene expression of NR on the transcriptional level (Rajasekhar et al., 1988). An indirect effect of light on the expression of NR genes is the result of carbohydrates such as sucrose, which are photosynthetic metabolites (Cheng et al., 1992). On the posttranslational level, NR regulation depends on photosynthesis through the balance of kinases and phosphatases, which control phosphorylation and dephosphorylation of the protein (Kaiser and Huber, 2001).

Nitrite content in vegetables can be minimized by balancing nitrate uptake and reduction (Demšar et al., 2004). For instance, in some growth environments, precise control of nitrate supply in accordance with the lighting conditions can be performed without loss in productivity. However, an alternative approach is to stimulate the reduction of nitrates by light. To that end, an attractive tool is solid-state lighting, which is based on narrow-bandwidth light-emitting diodes (LEDs) and offers possibilities in tailoring the illumination spectrum and controlling morphogenesis and productivity of plants (Folta and Childers, 2008; Massa et al., 2008; Morrow, 2008).

We report on the application of a solid-state illuminator for the reduction of nitrate content in leafy vegetables. The developed strategy involves several key points peculiar to solid-state lighting. First, our approach relies on short-term preharvest light treatment of leafy vegetables previously grown under usual high-pressure sodium lighting conditions. Compared with full-cycle growth, this implies economical viability of the proposed technology before the anticipated reduction of the capital cost of solid-state illuminators (Tamulaitis et al., 2005). Second, we use only narrow-bandwidth photosynthetic red light, which has the highest capacity in stimulating NR activity (Lillo and Appenroth, 2001). Blue light and far red light were avoided. Blue light is known to increase the overall nitrogen content (Ohashi-Kaneko et al., 2006) and to be less efficient for stimulation of nitrate reduction in matured plants (Maevskaya and Bukhov, 2004). Far red light reduces the rate of nitrate reduction (Johnson et al., 1999). Finally, we exploited the advantage of LEDs in the directional generation of high photosynthetic flux density (PPFD) without substantial exposure of leaves to radiant heat and consequent reduction of stomatal conductance.

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Materials and Methods

Plant material and growing conditions. Lettuce (*Lactuca sativa* cv. Grand Rapids), marjoram (*Majorana hortensis*, Moench.), and green onions (*Allium cepa*, L. cv. Lietuvos didieji) were grown in peat (pH $\approx$ 6, accuracy $\pm$ 0.01 pH units) substrate within an industrial greenhouse (April, Lithuania, lat. 55° N). The amount of nutrients (mg·L⁻¹) in substrate was as follows: nitrogen (N) 60–80, phosphorus (P) 30.5–50, potassium (K) 140–180, calcium (Ca) 200–300, magnesium (Mg) 40–60. Electrical conductivity (EC) varied between 1.0 and 2.5 m S/cm ($\pm$ 0.03 m S/cm). Plants were fertilized with 0.2% ammonium nitrate solution once a week. Additionally, lettuce was grown hydroponically (pH 5.5 to 6.0, accuracy $\pm$ 0.01 pH units). The nutrient composition of solution varied during vegetation period as follows: N 120 (nitrate nitrogen), P 50–70, K 220, Ca 100–120, Mg 80 (mg·L⁻¹). The EC varied between 1.2 and 1.7 m S/cm ($\pm$ 0.03 m S/cm). The growth up to harvesting time of the plants ($\approx$ 30 d) was performed under natural daylight (averaging $\approx$ 300 μ mol·m⁻²·s⁻¹ PPFD within a 14-h photoperiod and up to 1500 μ mol·m⁻²·s⁻¹ peak PPFD; daily integral of lighting $\approx$ 15 mol·m⁻²). Daylight was supplemented by high-pressure sodium lamps (HPS) (Son-T Agro; Philips, UK) at a PPFD of $\approx$ 130 μ mol·m⁻²·s⁻¹ (18-h photoperiod; daily integral of lighting $\approx$ 8.5 mol·m⁻²). At the preharvest stage of 3 d, plants were transferred to a phytotron chamber and exposed to either HPS lamps (reference) or a solid-state illuminator (treatment); a 24-h photoperiod was maintained in both cases. The temperature conditions in the phytotron were similar to those in the greenhouse (21 and 15 °C for 18 h and 6 h, respectively). The solid-state illuminator (Tamulaitis et al., 2005) contained high-power (3 W) red AlGaInP LEDs (LUXEON® III Star, Model LXHL-LD3C; Philips Lumileds Lighting Company, San Jose, CA) with the peak wavelength of 638 nm. The LEDs were mounted on an uncoated horizontal aluminum plate with the backside cooled by fans (such a design resulted in low radiant heat directed toward the plants). The LEDs were driven by a custom-made current regulator. The PPFD generated by the illuminator was maintained at 500 μ mol·m⁻²·s⁻¹. The PPFD was measured using a portable photosynthesis system (CI-310; CID, Inc., Camas, WA). Nutritional quality of the plants treated and reference plants were assessed after harvesting by measuring the concentrations of nitrate, carbohydrates, and vitamin C (L-ascorbic acid).

Assessment procedures and sample preparation. Nitrate concentration was measured by a potentiometric method (Geniatakis et al., 2003) using an ion meter (Oakton, IL) with a nitrate-selective electrode (Cole-Parmer, IL). Samples were prepared with 40 g fresh tissue per sample that was dried at 105 °C for 24 h and grounded. The ionic strength adjustor (ISA) contained 0.02 M Al₂(SO₄)₃, 0.01 M Ag₂SO₄, and 0.02 M H₃BO₃. The

weighted dry sample (0.2 g) was diluted with a 20 mL water–ISA solution (50/50% v/v). All measurements were performed after the sensor signal had been stabilized for 3 min.

Vitamin C was assessed by a spectrophotometric method of Janghel et al. (2007), which is based on the ability of the vitamin to reduce methyl viologen to stable free radical ion. Samples were prepared from 1 g plant material that was homogenized with 10 mL of 5% metaphosphoric acid and centrifuged at 2500 rpm for 5 min. Two milliliters of methyl viologen and 2 mL of 2 mol·L⁻¹ NaOH were mixed with a 1-mL sample extraction. After 2 min, absorption was measured at 600-nm wavelength using a spectrophotometer (Genesys 6; Thermo Fisher Scientific, Inc., Waltham, MA). The concentration was determined using the calibration data of ascorbic acid standards.

Carbohydrates (fructose, glucose, and maltose) were measured by a high-performance liquid chromatography (HPLC) method (Urbonavičiūtė et al., 2006). Samples were prepared from 1 to 2 g fresh tissue per sample that was ground and diluted with 4 mL of 75 °C double distilled water. The samples were prepurified using 0.2- μ m syringe filters. A HPLC system (10A; Shimadzu, Kyoto, Japan) equipped with a refractive index detector (RID-10A; Shimadzu) was used. Separations were performed on an Adsorbosil NH₂-column (150 mm by 4.6 mm; Alltech, IL). The mobile phase contained 75% acetonitrile and 25% double distilled water. The sensitivity of the HPLC method was established using a method validation protocol (ICH, 2005). The limits of detection and quantification were 0.01 and 0.03 μ g·mL⁻¹ for fructose, 0.12 and 0.27 μ g·mL⁻¹ for glucose, and 0.02 and 0.02 μ g·mL⁻¹ for maltose, respectively.

Chemicals. Acetonitrile, silver sulphate (Ag₂SO₄), and boric acid (H₃BO₃) were obtained from Poch (Gliwice, Poland). Al₂SO₄ and sodium hydroxide (NaOH) were purchased from DeltaChem (Povrly, Czech Republic). Fructose, glucose, maltose, and methyl viologen were obtained from Sigma-Aldrich (Steinheim, Germany). Ascorbic acid and metaphosphoric acid were acquired from Penta (Prague, Czech Republic) and Acros

Organics (Geel, Belgium), respectively. All standards and samples for HPLC were filtered through a 0.2- μ m syringe filters (Albet®, Sarstedt, Germany).

Statistical analysis. Data were processed using MS Excel software (Version 7.0; Microsoft Inc., Redmond, WA). A conjugated biological sample of the green matter of five plants randomly selected were used for each analysis. Three analytical replications of nitrates, vitamin C, and sugars were done for each treatment. SEs of means are indicated in figures by error bars ($P = 0.05$).

Results

In vegetable leaves exposed to light, generated by the solid-state illuminator, nitrate concentration exhibited a striking reduction by two to three times in comparison with those kept under HPS lamps (Fig. 1). The highest nitrate reduction rate was observed in hydroponically grown lettuce, in which the reference concentration of nitrate was highest as a result of easier inorganic nitrogen uptake from solution in comparison with solid substrate. After 3-d treatment under red LEDs, a relative decrease of nitrate concentration by 65% was achieved for these plants. The relative decrease was very similar in all species (from 44% to 65%) and was almost independent of the reference nitrate concentration that varied from 480 mg·kg⁻¹ in green onions to 4270 mg·kg⁻¹ in hydroponic lettuce.

All species treated under high PPFD exhibited higher net concentration of carbohydrates in comparison with the reference plants (Fig. 2). The highest increase in net carbohydrate concentration was observed in peat-grown lettuce (by more than 300%) and marjoram (by $\approx$ 60%). Note that the major share of the net concentration increase is the result of an increase of concentration of monosaccharides (fructose and glucose), which are of higher nutritional value in comparison with disaccharides (maltose).

In contrast with carbohydrates, no correlation of vitamin C content with the stimulation of nitrate reduction was observed (Fig. 3). In onion leaves, the concentration of vitamin C increased by 25% with high PPFD. In marjoram and hydroponic lettuce, it remained

Fig. 1. Nitrate concentration (fresh mass) in leafy vegetables grown only under high-pressure sodium lamps (left columns) compared with those subjected to 3-d preharvest treatment under red light-emitting diodes (right columns). Error bars indicate the SE of means ($P = 0.05$).

Fig. 2. Carbohydrate concentration (fresh mass) in leafy vegetables grown only under high-pressure sodium lamps (left columns) compared with those subjected to 3-d preharvest treatment under red light-emitting diodes (right columns). Error bars indicate the SE of means ($P = 0.05$).

Fig. 3. Vitamin C amount (fresh mass) in leafy vegetables grown only under high-pressure sodium lamps (left columns) compared with those subjected to 3-d preharvest treatment under red light-emitting diodes (right columns). Error bars indicate the SE of means ($P = 0.05$).

almost unchanged, whereas in peat-grown lettuce, the concentration dropped by 46%.

Discussion

The results of our study indicate that nitrate content in lettuce, marjoram, and green onions can be considerably reduced by several times using short-term (3 d) preharvest treatment under purely red light with high PPFD (Fig. 1). Note that the use of narrow-bandwidth red LEDs assembled into an illuminator with low radiant heat allows effective application of much higher PPFDs than those under conventional discharge or fluorescent lamps. An essential feature of our approach is in that the production volume of leafy vegetables can be grown under usual HPS lighting conditions typical of greenhouses in northern countries, whereas the health quality can be improved within a relatively short treatment using an advanced solid-state illuminator. Because the treatment is conducted only over 10% of the overall growth cycle, the capital cost limitations for the application of solid-state lighting in horticulture are mitigated.

Our results (Fig. 1) indicate that the increase of nitrate reduction rate under high-PPFD red light in comparison with the HPS treatment may be proportional to the initial concentration of nitrate. This is in line with the fact that the reduction is the result of

a combined action of the presence of nitrate and red light on the NR expression and posttranslational regulation (Crawford, 1995; Lillo and Appenroth, 2001).

The observed drop in nitrate concentration (Fig. 1) coincides with an increase in concentration of carbohydrates (Fig. 2). The increase in concentration of carbohydrates is probably the result of the increased photosynthesis intensity in response to intense red light. Besides, physiologically active phytochrome (P_{fr} isoform) may also have a role in increased release of soluble carbohydrates resulting in a concurrent decrease in leaf nitrate levels (Schittenhelm et al., 2004). The increase in carbohydrate concentration is favorable in terms of both nutritional quality and self-supporting NR activity through stimulation of gene expression (Cheng et al., 1992; Lillo and Appenroth, 2001). Moreover, additional stimulation of expression of genes involved in nitrogen assimilation can be caused by a joint action of carbohydrates and light (Thum et al., 2003). As was already noted, the increase in net carbohydrate concentration is attributable mainly to an increase in monosaccharide content. This is also important in view of nitrate reduction, because monosaccharides such as glucose can have a greater effect in regulating nitrogen metabolic genes than nitrogen itself (Price et al., 2004).

The observed alternations in vitamin C concentration (Fig. 3) indicated an absence of a direct correlation of its metabolism with the reduction of nitrate. However, the tendency of dropping the vitamin C content in some LED-treated species deserves discussion, because vitamin C is not only an important nutrient, but also plays a major role in the protection of plants against photo-oxidative stress, photoprotection, and phytohormone action (Conklin, 2001; Davey et al., 2000; Pastori et al., 2003). One of the possible reasons for the decline in synthesis of ascorbic acid is leaf aging. Interestingly, a noticeable drop in vitamin C content is observed in peat-grown lettuce in which high-PPFD treatment invoked a striking increase in the concentration of carbohydrates (Fig. 2), which are known to trigger leaf ageing (Wingler et al., 2006). This implies that care against overmaturing of plants resulting from intense photosynthesis might be important to take when treating plants under high PPFD.

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