**Effects of LED lighting spectra on lettuce (*Lactuca sativa* L. ‘Frillice’) growth and nutritional composition**

**T Hytönen** PhD a, **P Pinho** DSc b,[[1]](#footnote-1)\*, **M Rantanen** a, **S Kariluoto** PhD c, **A Lampi** PhD c, **M Edelmann** PhD c, **K Joensuu** a, **K Kauste** a, **K Mouhu** PhD a, **V Piironen** PhD c, **L Halonen** DSc b, **P Elomaa** PhD a

a Department of Agricultural Sciences, Viikki Plant Science Centre, University of Helsinki, P.O. Box 27, FI-00014 University of Helsinki, Finland

b Department of Electrical Engineering and Automation, Aalto University, P.O.Box 13340, FI-00076 AALTO, Finland

c Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, FI-00014 University of Helsinki, Finland

ABSTRACT

**Year-round greenhouse production in northern latitudes depends on the use of artificial lighting. Light emitting diodes (LEDs) provide a promising alternative to save energy during cultivation as well as to modify the light spectrum to regulate the growth and quality of the crop. We compared the effects of LED lighting with different spectral compositions on lettuce growth and development as well as on nutritional quality. We show that warm-white and warm-white supplemented with blue spectra provides equal growth and product quality compared to conventional HPS lighting in the absence and presence of daylight. Our data indicates that for biomass accumulation the far-red component in the light spectrum is more critical than green light or red/blue ratio. Furthermore, we demonstrate that red+blue spectrum increases the concentration of several vitamins in lettuce. However, biomass accumulation using this spectrum was insufficient when daylight was excluded.**

**Keywords:** artificial lighting, biomass, greenhouse production, nitrate, vitamin, LED lighting

# INTRODUCTION

Artificial lighting is necessary for year round greenhouse production of horticultural crops in northern latitudes. During the last decades, high-pressure sodium (HPS) lamp has become the major horticultural light source, because of its high photon flux emission, low cost, long operational life time, and high electrical efficiency 1. The electrical energy consumption due to artificial lighting significantly increases the production costs especially during the winter. These high costs and increasing demand for energy savings require the development of more energy-efficient lighting technologies. The light emitting diode (LED) is a promising and fast-developing technology that is expected to provide significant energy savings in various applications in the near future. These include development of novel LED regimes for horticultural lighting 2,3.

For plants, light is essential as it provides energy to drive photosynthesis. However, light also acts as an important external source of information that affects plant growth and development. Chlorophylls are major photosynthetic pigments, and chlorophyll molecules isolated in an organic solvent absorb blue and red light. Therefore, these wavebands are thought to support plant growth most efficiently 4. Other photoreceptors, including blue light receptors cryptochrome and phototropin as well as red/far-red absorbing phytochromes, monitor light spectra to control growth and development, and consequently, the phenotype of the plant. The regulation of stem elongation, leaf expansion, flowering, opening of the stomata and phototropism are examples of developmental responses mediated by these receptors 5. Also green light has been shown to affect plant growth and development since a relatively high proportion of it is transmitted through leaves and penetrates deeper into the canopy 6,7.

Although HPS lamps have been successfully used in horticultural lighting, their light emission, which is predominantly in the green and yellow regions of the electromagnetic spectrum, is not optimal for photosynthesis. For example, cucumber plants grown solely under HPS lamps without natural light remain stunted and accumulate less dry mass compared to plants grown under artificial solar spectrum 8. In contrast, colour LEDs, which can emit light in a narrow-bandwidth wavelength region, can be used to tailor the spectrum in order to optimize photosynthesis and to regulate the growth and development of the crop 7,9,10. In earlier studies, mostly red and blue LEDs have been compared to conventional lamp types but also green and far-red LEDs have been tested 11,12. Plants are able to grow with red light only, but the addition of blue light up to 50% of total photosynthetically active radiation (PAR) has been shown to increase the photosynthetic capacity, and consequently, biomass accumulation 10,13,14. Moreover, blue light has been shown to reduce the elongation growth, while far-red light has an opposite effect 9,15,16. Also supplementing red + blue spectrum with green light as well as the addition of far-red to the red light has been shown to increase growth and biomass accumulation of lettuce seedlings 7,17. LED technology allows us to receive more comprehensive knowledge on the effects of light spectrum on growth and development of diverse greenhouse crops, which is necessary for more controlled production to meet the requirements of the market.

Light also has a role in regulating secondary metabolism of plants. Green leafy vegetables are good dietary sources of many vitamins. Folate, one of the B vitamins, exists as several vitamers that mediate the transfer of one-carbon units in various metabolic processes (C1-metabolism). Typically, 5-methyltetrahydrofolate dominates in plants, whereas 5-formyltetrahydrofolate might serve as a storage form and can convert to 5,10-methenyltetrahydrofolate 18. In humans, adequate folate intake is known to prevent neural tube defects and megaloblastic anaemia and may reduce the risk of other birth defects, cardiovascular disease, several cancer types, and cognitive disorders 19. Lettuce is widely consumed and abundant in folate 20,21. Folate synthesis is promoted by light but the association between folate and photosynthesis as well as related metabolism is not yet understood 18. It has been shown that storage under continuous light may increase folate contents in spinach 22. However, little is known about the effect of light intensity or spectrum on folate in greenhouse conditions.

Tocopherols, including active vitamin E α-tocopherol, and carotenoids, including vitamin A provitamins, are antioxidant compounds that are synthesized in photosynthetic organisms from the isoprenoid biosynthetic pathway 23. They are accumulated in thylakoids, envelopes and plastoglobuli in chloroplasts 24, and are present in high amounts in green vegetables. In plants, the major function of carotenoids and tocopherols is to protect photosynthetic membranes from oxidative stress. During high-intensity light stress, chlorophylls are excited and reactive oxygen species are formed increasing the need for antioxidant protection. In general, total carotenoid levels are higher in high-light and sun leaves than in low-light and shade leaves 24. The level of α-tocopherol steadily increases in leaves under high-intensity light stress, and several-fold levels may be found in late summer compared to spring 24,25. To our knowledge, the effect of light spectra on these vitamins has not yet been studied.

Since high intake of nitrate causes risks for human health, European Union Commission has established regulations for the maximum nitrate levels for vegetables 26. Green leafy vegetables typically have high nitrate content, which may exceed the limits set by EC. Earlier studies have shown that nitrate content in lettuce plants correlates negatively with photosynthetic activity 27, and therefore nitrate concentration increases with decreasing light intensity 28. However, a more recent study revealed that nitrate concentration in leafy vegetables can be substantially reduced by short-term pre-harvest treatment with red LED lighting 29.

The aim of this study was to compare the effect of light spectrum on lettuce (*Lactuca sativa* L.) growth, morphology and accumulation of phytonutrients. Four different LED lighting spectra (red + cool white, RCW; red + blue, RB; warm white, WW and warm white + blue, WWB) were firstly evaluated in the absence of daylight. Finally, the LED spectrum which provided optimal biomass accumulation in the absence of daylight was tested as a supplemental light to daylight. In both experiments, conventional HPS lamps were used as control.

# MATERIALS AND METHODS

### **Plant material and growing system**

Pilled lettuce seeds of cv. Frillice were sown in a coarse growing peat (Kekkilä Oyj, Tuusula, Finland). Two plants per pot were allowed to grow. Plants were transferred into hydroponic system when they had approximately two growing leaves and grown at a density of 45 plants per m2. Complete nutrition solution (Vihannes-Superex, 9-5-31 NPK, Kekkilä, Finland) with electrolyte conductivity (EC) of 1.8 was used. EC value was monitored twice a week. Growing temperature was set to 17.5/16.5 ± 1.0 °C (day/night) and daily photoperiod was 20 h. Two experiments were carried out at the University of Helsinki with different lighting systems described below.

In experiment 1, one treatment plot of approximately 2 × 2.5 m was constructed for each lighting system (see below). Plots were surrounded by non-transparent reflective plastic film (walls, height 2 m). In addition, natural light was excluded with similar plastic film above the plastic walls (roof). The walls and the roof were separated by a distance of 0.5 m to allow proper air circulation between the treatment plots. Twenty plants were grown in the middle of each treatment plot, and these plants were surrounded with additional plants in all sides in order to avoid border effects. At the end of experiment, five plants per treatment were randomly selected for nutritional analysis and fifteen plants were used for growth observations (see below).

In experiment 2, non-transparent reflective plastic films were set in the both sides of the two treatment plots in north-south direction with the distance of four meter, and HPS or WW LED lamps were set in the middle of the plot. This setup excluded light from another treatment, but allowed the efficient use of natural light coming to the greenhouse through the roof during the day. Twenty four plants surrounded by additional plants on all sides were grown in both plots. Four randomly selected plants per treatment were used for nutritional analysis and 20 plants for growth observations.

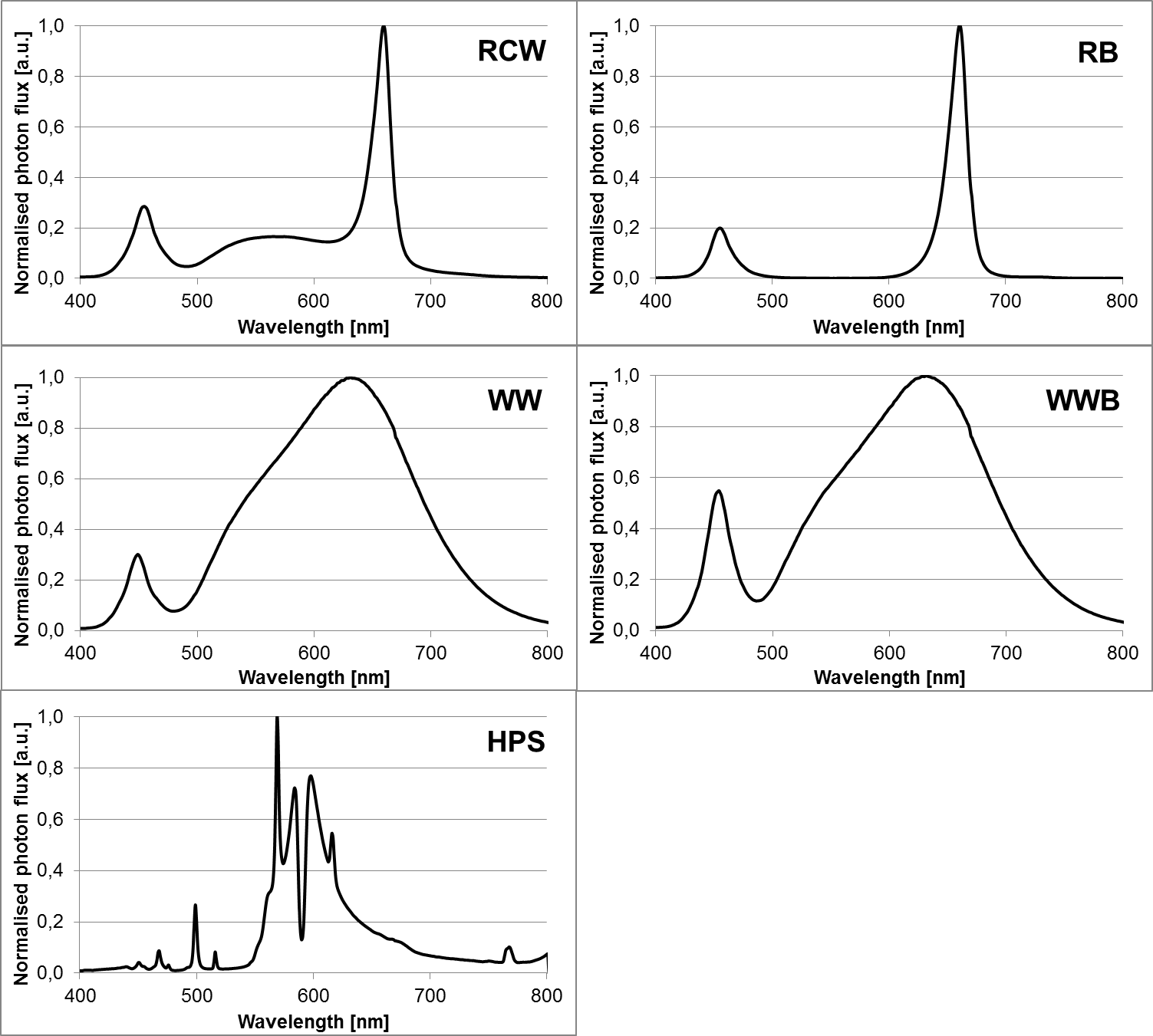
### **Lighting systems**

### In experiment 1, the four LED lighting spectra RCW, RB, WW and WWB were compared to HPS lighting. Experiment was carried out in March-April 2010. The LED luminaires were composed by arrays of 6-W red, blue, cool-white and 13-W warm-white LEDs modules (Light Line Source L-CM12/L-CM6, Citizen Electronics Co. ltd, Japan). The peak wavelengths of the red and blue LEDs were 660 nm and 450 nm, respectively. The correlated colour temperature (CCT) of the warm-white and cool-white LEDs was 2700 K and 5000 K, respectively. HPS lamps (PLANTASTAR 400 W, Osram, Germany) were installed in two high-bay luminaires (Cropmaker, Elektro-Valo Oy, Finland).

### The normalized spectral photon flux distribution of the lighting systems (Fig. 1) were determined based on the spectral power distribution measured with a calibrated Spectral Lamp Measurement System (SLMS, Optronic Laboratories, USA) consisting of an Ulbricht-type integrating sphere with a thermoelectrically cooled diode array spectrometer, computer and LabScan software. In order to calculate red to blue (R/B), red to far-red (R/FR), and green to blue (G/B) ratios (Table 1), blue, green, red and far-red spectral components were determined from the integration of the photosynthetic photon flux between 400 – 500 nm, 500 – 600 nm, 600 – 700 nm, and 700 – 800 nm wavelength ranges, respectively. The average photosynthetic photon flux density (PPFD) was determined for each lamp (Table 1) based on measurements on 81 points uniformly distributed over the growth area at plant canopy height using a high-resolution spectrometer (HR4000, Ocean Optics, USA).

### Table 1. Photosynthetic photon flux density (PPFD) within PAR spectral region (i.e., 400 – 700 nm), and red (600 – 700 nm) to blue (400 – 500 nm), red to far-red (700 – 800 nm), and green (500 – 600 nm) to blue photon flux ratios of lighting systems. PPFD values are averages of 81 measurements in the growing area ±SD. Lighting systems were RCW = red + cool white; RB = red + blue; WW = warm white; WWB = warm white + blue; HPS = high pressure sodium.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | RCW | RB | WW | WWB | HPS |
| PPFD | 134±17 | 131±10 | 128±18 | 136±20 | 144±6 |
| R/B ratio | 3.1 | 4.5 | 7.5 | 4.4 | 9.1 |
| R/FR ratio | 22.6 | 76.3 | 4.9 | 4.9 | 4.3 |
| G/B ratio | 1.5 | 0.0 | 4.9 | 2.9 | 9.0 |



### Figure 1. Spectral photon flux distribution of the lighting systems used in this study. RCW = red + cool white; RB = red + blue; WW = warm white; WWB = warm white + blue; HPS = high pressure sodium.

### In experiment 2, the effect of WW LEDs and HPS lamps on lettuce growth and quality were evaluated in the presence of daylight. Experiment was carried out in September-October 2010. The PPFD due to supplemental lighting was 152±11 and 157±12 µmol m–2 s–1 under WW LED and HPS lamps, respectively. Lamps were conventionally controlled using discontinuous on-off regime. Supplemental lighting systems were switched off when the outside global solar irradiance exceeded the 270 W m-2 threshold level. This regime was implemented using the greenhouse climate control installation (Intégro, Priva, The Netherlands). The average photosynthetic photon flux integral was approximately 14 mol m–2 per day in both treatments which corresponds to a constant PPFD of about 200 µmol m-2 s-1 during the 20 h lighting period.

**Growth and external quality observations**

After five weeks of growth period, height of the plant, plant leaf area (only in experiment 1), number of leaves, and plant fresh and dry weight excluding roots were recorded. Tip burn was evaluated based on the qualitative scale where 0 = no visible tip burn, 1 = some symptoms visible, 2 = severe symptoms, and 3 = not commercially acceptable. Observations were performed in 15 or 20 plants in experiments 1 and 2, respectively.

### **Nutritional value**

Five or four individual plants were randomly sampled for chemical analyses in experiment 1 and 2, respectively. Whole plants were immediately frozen in liquid nitrogen and vacuum dried in darkness (Heto drywinner FD 8, Cambridge Biosystems, UK) and folate, carotenoids, tocopherols, and nitrate were determined.

Total folate contents were determined in duplicate by microbiological assay on microtiter plates using *Lactobacillus rhamnosus* ATCC 7469 as the test organism. Sample preparation included heat extraction followed by trienzyme treatment with hog kidney conjugase, α-amylase, and protease as reported previously 30. Method performance was confirmed by analysing a blank sample as well as certified reference material BCR 485 (Mixed vegetables; obtained from the Institute for Reference Materials and Measurements, Geel, Belgium) in each set of samples. Folate vitamers were quantified in duplicate by ultra-high performance liquid chromatography (UHPLC) after affinity chromatographic purification as described by Edelmann et al. (2012) 31.

Lutein as the major carotenoid was analysed by reversed-phase high-performance liquid chromatography (RP-HPLC) using an internal standard method according to Caldwell and Britz (2006) 32. In brief, freeze-dried samples, ca 10 mg, were extracted with 1 mL cold 80% acetone with 0.01% butylated hydroxytoluene and 5 µg of -apo-8’-carotenal (internal standard, Sigma, USA) by vortexing for 1 min. After the extract was withdrawn, the residue was re-extracted with 1 mL of the same solvent except for the internal standard, and the extracts were combined. Filtered extracts (0.45 µm, GHP Acrodisc 13) were analyzed by RP-HPLC with UV detection set at =412 nm using an octadecylsilica column (5 µm, 4.6 × 250 mm, Zorbax ODS, Agilent, USA) and gradient elution with 80% aqueous methanol with 0.05% triethylamine and ethylacetate with 0.05% triethylamine at 1 mL min-1 at 24 C 32. Quantification was based on linear calibration curves of mixtures of lutein (in ethanol: =444 nm, E1%=2550) and -apo-8’-carotenal (in ethanol: =457 nm E1%=2640) with actual concentrations measured by UV. Method performance was confirmed by analysing in each extraction batch the same certified reference material BCR 485 as used in folate analysis. Lutein content of the reference material was 13.90.6 µg g-1 (mean  stdev; n = 13) whereas the certified value was 12.5 µg g-1 DM with an uncertainty of 0.8 µg g-1 DM. Recovery of added lutein at 3 µg/sample level was 92% (n = 5). Each lettuce sample was analyzed in triplicate.

Tocopherols, -, -, and -vitamers, were analyzed by normal-phased high-performance liquid chromatography (NP-HPLC) using an internal standard method. Tocopherols were extracted and prepared for HPLC analysis as carotenoids except for using heptane as the solvent and -tocopherol (0.5 µg) as an internal standard (No 15496, für biochemische Zwecke, Merck, UK). Tocopherol analysis was done according to Schwartz et al. (2008) 33 using a silica column (5 µm, 4.6 × 250 mm, Inertsil, Varian Inc., Torrance, USA) and 3% 1,4-dioxane in heptane elution with 2 mL min-1 and fluorescence detection (ex=292 nm, em=325 nm) at 30 C. Quantification was based on linear calibration curves of mixtures of - (in ethanol: =292 nm, E1%=75.8), - (in ethanol: =298 nm, E1%=91.4), - (in ethanol: =298 nm, E1%=87.3), and -tocopherol (in ethanol: =296 nm E1%=89.4) with actual concentrations measured by UV. Recovery of added - and -tocopherols at 1 µg/sample level were 106% and 95% (n = 5), respectively. Each lettuce sample was analyzed in triplicate.

Nitrates were eluted from freeze-dried samples by milli-Q-water. Nitrate content was analysed spectrophotometrically in a commercial analytical lab (Soil service analysis, Mikkeli, Finland).

Chlorophyll A and B and total chlorophyll levels were measured from frozen non-dried samples as described earlier 34.

**Experimental design and statistical analyses**

Both experiments were carried out as completely randomized experiments in a single greenhouse room. Plants were randomly assigned to the treatments (different light spectra, k = 5 for experiment 1 and k = 2 for experiment 2). The number of replicates (individual plants) was 15 or 20 for growth measurements and 5 or 4 for nutritional analyses in experiments 1 and 2, respectively. In experiment 1, the growth, vitamin and nitrate data were subjected to analysis of variance and pairwise comparisons of the treatments (LED spectra) to the control (HPS) were performed using Dunnett’s test at the significance level α = 0.05. In experiment 2, two-independent-sample comparisons between the treatments were performed using Student’s t-test. Statistical analyses for the data were carried out with the SAS statistical software package (SAS/STAT software, version 9.2 of the SAS System for Windows, SAS Institute Inc. Cary, NC, USA). The presence of tip-burn was analyzed with the non-parametric Kruskal-Wallis test and the pairwise comparisons to control treatment were carried out separately with the Mann-Whitney U test with Bonferroni correction at the significance level α = 0.05.

# RESULTS

**Lighting spectra affected lettuce growth and biomass accumulation**

In experiment 1, which was carried out without natural light, light quality significantly affected the shoot fresh and dry weight, the dry matter content, the height, the number of leaves and the leaf area of lettuce plants (*p* < 0.001 for all variables). Almost equal shoot fresh weight was found in the plants grown for five weeks under HPS, WW and WWB lighting, whereas the fresh weight of plants grown under RCW or RB LEDs was over 40% less than the weight of the plants in the HPS treatment (Fig. 2A, B). Similarly, the dry weight of RCW and RB grown lettuce plants was smaller than in the control plants. Furthermore, plants grown under RCW and RB lamps were shorter than plants under HPS whereas WW or WWB spectra did not affect elongation growth (Fig. 2C). Consistent with higher fresh weight and elongation growth, the leaf area was also larger in the plants grown under HPS lamps than in plants grown under RCW or RB conditions, whereas the leaf area of plants grown under WW and WWB lamps did not differ from HPS (Fig. 2D). The number of leaves was higher in HPS treatment than in all other treatments (Fig. 2E). Overall, the size of the plants exceeded the weight and height limits of marketable pot lettuce in Finland (100 g and 13 cm, respectively) only in HPS, WW and WWB treatments during 5-week growing period. The plant size variation between the treatments did not correlate with changes in chlorophyll contents, since no statistically significant differences were found in ChlA, ChlB or total chlorophyll contents between LED and HPS treatments (data not shown).



Figure 2. The effect of lighting spectra on lettuce growth (A), fresh weight (B), plant height (C), total leaf area (D) and the number of leaves (E) in experiment 1. Asterisk above the bar indicates significant difference compared to HPS treatment according to Dunnett’s test (α = 0,05; df 4, 70). Error bars indicate ±SE. RCW = red + cool white; RB = red + blue; WW = warm white; WWB = warm white + blue; HPS = high pressure sodium.

In experiment 2, HPS lamps and WW LEDs were tested as a supplemental light. In this experiment, the average fresh weight exceeded 200 g in both treatments during 5-week growing period, but no statistically significant differences between treatments were found in any of the growth parameters analysed (data not shown).

**Tip-burn symptoms were observed in all treatments**

Tip-burn, i.e. browning of the leaf edges caused by calcium deficiency 35, is a common problem in greenhouse grown lettuce. We evaluated the presence of tip-burn in the plants grown under different lighting spectra by using 0 – 3 scale. All plants grown under RCW and RB spectra had tip-burn symptoms, and severe tip-burn symptoms (class 2) were found in almost half of the plants (Fig. 3). In the plants grown under WWB spectra, no class 2 symptoms were observed and in WW and HPS treatments they were rare. In addition, in WW, WWB and HPS treatments, some plants had no tip-burn at all. However, differences in the presence of tip-burn between HPS and LED lighting treatments were not statistically significant. In experiment 2, tip-burn was present in all plants, and about half of the plants had class 2 tip-burn symptoms in both HPS and WW treatments (data not shown).



Figure 3. The presence of tip burn in the lettuce plants grown under different lighting spectra. The percentage of plants in the each class is shown. Class 0 = no symptoms, class 1 = some symptoms, class 2 = severe symptoms. *n* = 15. RCW = red + cool white; RB = red + blue; WW = warm white; WWB = warm white + blue; HPS = high pressure sodium. According to the non-parametric Kruskal-Wallis test, lighting treatments had statistically significant effect on the presence of tip-burn (*p* < 0.001; df 4,70). However, comparison of LED treatments with the control (HPS) did not reveal statistically significant differences (Mann-Whitney U test with Bonferroni correction; α = 0.05).

**Lighting spectra affected vitamin and nitrate contents**

In experiment 1, lighting treatments affected total folate contents (*p* = 0.013) which ranged from 12.4 to 15.0 µg g-1 DM between the treatments according to the results of the microbiological method (Table 2). The comparison of different LED treatments with HPS revealed that folate content was about 20% higher in plants grown under RB, but no statistically significant differences were found between other LED treatments and HPS according to Dunnett’s test. Results obtained by UHPLC correlated well with the microbiological method: the sum of vitamers was in average 89±10% of the microbiologically determined total folate content. The content of major vitamer, 5-methyltetrahydrofolate (5-CH3-H4), was 46 – 67% of the vitamer sum, but differences between HPS and LED treatment were not statistically significant (Table 2). The proportion of 5,10-methenyltetrahydrofolate (5,10-CH+–H4) varied from 23 to 39% of the vitamer sum, and plants in the RB and RCW treatments accumulated significantly more 5,10-CH+-H4 than plants grown under HPS lamps. No differerences were found in the 5-formyltetrahydrofolate (5-HCO-H4) and tetrahydrofolate (H4) contents which accounted for less than 10% in all samples. The level of 10-formylfolic acid (10-HCO-PGA) and folic acid (PGA) were very low and these vitamers were not detected in all samples (data not shown). Total folate contents were similar in experiments 1 and 2 (HPS and WW) showing good repeatability (Table 2). Also in the second experiment, 5-CH3-H4 and 5,10-CH+-H4 were the most abundant vitamers. However, comparable levels of different vitamers were found in HPS and WW treatments.

Table 2. Folate, tocopherol, lutein and nitrate contents (μg g-1 DM; mean ±SE) in plants grown under different lighting spectra. Plants were grown without or with natural light in experiments 1 and 2, respectively. Asterisk after the value indicates statistically significant difference compared to HPS treatment in experiment 1 (Dunnett’s test; α = 0.05; df 4, 20) and experiment 2 (Student’s t-test; df 6). Total folate content was measured by microbiological method, folate vitamers by UHPLC, and tocopherols and lutein by normal- or reversed-phased HPLC, respectively. RCW = red + cool white; RB = red + blue; WW = warm white; WWB = warm white + blue; HPS = high pressure sodium.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Experiment 1 | | | | | Experiment 2 | |
|  | RCW | RB | WW | WWB | HPS | WW | HPS |
| Total folate | 13.8±0.2 | 15.0±0.8\* | 13.0±0.6 | 12.4±0.3 | 12.4±0.6 | 12.9±0.3 | 12.4±0.5 |
| 5,10-CH+-H4 | 4.2±0.2\* | 4.6±0.2\* | 3.4±0.2 | 2.9±0.1 | 3.2±0.3 | 3.0±0.5 | 3.2±0.4 |
| 5-HCO-H4 | 0.8±0.1 | 0.9±0.0 | 0.8±0.1 | 0.7±0.0 | 0.9±0.1 | 0.5±0.1 | 0.5±0.1 |
| 5-CH3-H4 | 5.1±0.1 | 5.5±0.4 | 5.4±0.2 | 6.8±0.2 | 6.1±0.4 | 8.7±0.8 | 7.6±0.4 |
| H4 | 0.6±0.0 | 0.7±0.1 | 0.6±0.0 | 0.6±0.1 | 0.7±0.1 | 0.3±0.0 | 0.2±0.0 |
| Total tocolpherols | 314±4 | 358±8\* | 284±10 | 292±22 | 277±12 | 277±18 | 269±14 |
| α-tocopherol | 66±2 | 73±3\* | 62±2 | 67±5 | 60±3 | 67±6 | 62±3 |
| γ-tocopherol | 236±3 | 272±6\* | 212±9 | 216±18 | 208±10 | 200±11 | 197±11 |
| δ-tocopherol | 12.3±0.3\* | 13.2±0.2\* | 10.8±0.4\* | 9.0±0.5 | 9.3±0.3 | 10.3±0.5 | 9.3±0.4 |
|  |  |  |  |  |  |  |  |
| Lutein | 538±11 | 615±23\* | 446±29 | 450±25 | 459±20 | 399±20 | 370±25 |
|  |  |  |  |  |  |  |  |
| Nitrate | 73±2\* | 78±2 | 88±3 | 88±3 | 85±2 | 103±4 | 113±6 |

The light quality also affected total tocopherol contents in lettuce in experiment 1 (*p* = 0.002) (Table 2). Significantly more total tocopherols were found in RB treatment than in the control. -tocopherol was the major tocopherol in all samples. Its proportion ranged from 72 to 76% of total tocopherol, and -tocopherol contributed to 19 to 24%. Moreover, the level of δ-tocopherol was low in all treatments. The comparison of tocopherol levels in the plants grown under different spectra revealed that RB spectra increased the contents of - and -tocopherols compared to the control. In addition, higher δ-tocopherol levels were found under RCW, RB and WW spectra than in the HPS treatment. Total tocopherol and lutein contents were closely related to each other and showed a positive correlation coefficient of 0.956. Thus, lutein contents were also the highest in lettuces grown under RB lamps whereas no differences were found between HPS and other lighting spectra. The absence or presence of natural light in experiments 1 and 2, respectively, did not affect total tocopherol contents. However, lutein accumulation was slightly increased when natural light was excluded.

The analysis of nitrate content revealed that plants grown under RB, WW or WWB spectra accumulated similar amounts of nitrate than plants illuminated with HPS lamps in experiment 1, which was carried out without natural light (Table 2). However, significantly less nitrate was found in the plants grown under RCW lamps compared to the HPS treatment. When WW LEDs and HPS lamps were used as a supplemental light in experiment 2, clearly higher nitrate levels were found compared to experiment 1 (Table 2). However, no differences were found between the treatments.

# DISCUSSION

LEDs are promising light sources for the cultivation of greenhouse crops, since they allow optimization of the light spectra and have a potential to reduce energy consumption 2,36. In this paper, we have analyzed the effect of several LED spectra on various growth and quality parameters of greenhouse grown lettuce cv. Frillice. Equal biomass accumulation was found under WW and HPS spectra, whereas significantly lower yield was observed under RCW and RB LED lights. Since we found only minor differences in the number of leaves, the differences in the biomass accumulation were mostly due to observed changes in the plant height and leaf area. In addition, light spectrum is known to affect leaf thickness 37. However, we did not find differences in the specific leaf area (data not shown) which is a good estimation of leaf thickness.

Biomass accumulation is dependent on photosynthesis driven by the light absorption of chlorophyll pigments, which have absorption peaks in blue and red wavelengths of the light spectrum 4. We found that RB light spectrum provided insufficient growth results compared to HPS lamps, indicating that additional wavelengths are needed to enhance biomass accumulation. In strong white light, green light has been shown to enhance photosynthesis more efficiently than red light because it penetrates deeper in the leaves and can be absorbed by chlorophyll molecules at lower cell layers 38. Furthermore, the addition of 24% of green light into the RB spectra has been found to increase leaf area and fresh weight of lettuce seedlings 7, but this was not the case in our study. We found that similarly to RB light, RCW spectrum that contains approximately 27% of green light (500 – 600 nm) was poor in terms of biomass accumulation compared to HPS spectrum. One possible explanation for different results is a difference in green light spectra: Kim et al. (2004) used lamps that emitted a more pronounced green band between 500-550 nm 7, whereas RCW lamps had higher emission between 550-600 nm. This idea is supported by the finding that small changes in the bandwidth of green light have a significant effect on lettuce growth 39. It is also important to notice that HPS, WW, and WWB spectra that provided the highest biomass accumulation had the highest G/B ratios (Table 1). We also calculated R/B ratios of our luminaires and found that HPS lamp had two or three times higher R/B ratio than our RB or RCW spectra, respectively (Table 1). Since B light is known to reduce elongation growth 15,16, low level of B light in HPS lamps is one possible reason for taller plants observed under this spectrum. However, also WWB LED had low R/B ratio compared to HPS lamps, but equal growth was observed under HPS and WWB lamps.

Calculation of R/FR ratios revealed large differences between the spectra used in this study. Poor growth results in RB and RCW correlated with very high R/FR ratios compared to HPS lamps, whereas WW and WWB spectra, which promoted biomass accumulation comparable to HPS lamps, had similar R/FR ratios with HPS (Table 1). Therefore, slow biomass accumulation under RCW and RB spectra is likely to be directly related to the lack of photon emission in the FR region. It is known that increasing FR radiation relative to R promotes leaf expansion in some plant species 9,40. Consistent with this, significantly smaller leaf area was found in RB and RCW treatments compared with HPS, whereas no differences were found between HPS and WW or WWB suggesting that slower biomass accumulation in RB and RCW was associated with decreased leaf expansion. In conclusion, our results suggest that for cultivation of lettuce plants in the absence of daylight, the FR spectral component is more critical for efficient biomass accumulation than R/B ratio, but the role of green light in the white light spectra requires further studies. Additional experiments are also needed to reveal the importance of supplemental FR light in the greenhouse production of lettuce in northern latitudes during winter, when the intensity of natural light is low. Furthermore, the effect of additional blue light in the presence of FR light should be tested, since relatively high level of blue light is needed to enhance photosynthetic efficiency 10. However, a decrease in the elongation growth caused by blue light has to be taken into account 16,41. Under ideal conditions, lighting spectra could be tailored according to seasonal changes in the natural light considering the requirements of the plant and the energy efficiency of different LEDs.

We also studied the nutritional quality of lettuce plants grown in different lighting spectra by analyzing their vitamin and nitrate contents. The total folate, tocopherol and lutein contents fell to the range observed in different lettuce cultivars in previous studies 20,21,42,43. We found that RB spectrum caused a general increase in the vitamin contents compared with HPS spectrum indicating that this lighting spectrum can potentially be used to improve nutritional quality of lettuce plants grown without natural light. However when calculated per plant, the total vitamin contents were not increased under RB spectra because of the slower growth rate in this treatment compared to HPS. In contrast to RB spectrum, vitamin contents in other LED treatments were mostly comparable with HPS control although RCW tended to slightly increase vitamin levels. To our knowledge the effect of light spectra on folate and tocopherol contents has not been analyzed previously, but a few studies on carotenoids have been carried out. Li and Kubota (2009) showed that supplementing white with blue light increased carotenoid contents by 12 % 44. In addition, supplemental UV-A and UV-B lights had varying effects on carotenoids in eight varieties of green leaf lettuces 32.

RB and RCW spectra slightly affected the content and proportion of different folate vitamers compared to HPS. In our study, 5‑methyltetrahydrofolate was the dominant folate vitamer, as reported in leafy vegetables in other studies 45,46. However, according to Johansson et al. (2007), in lettuce cultivars formylated vitamers generally dominate over 5-methyltetrahydrofolate 21. They did not report any 5,10‑methenyltetrahydrofolate in cv. Frillice, although it was the second most abundant vitamer in our study. Instead, they found larger proportion of 5-formyltetrahydrofolate. These differences in the vitamer distribution pattern may be related to different post-harvest conditions, sample storage, and pretreatments as well as the analytical method 21,47. In contrast to folate vitamers, although RB spectrum increased total tocopherol content, light quality had no effect on the proportion of different tocopherols. -tocopherol was the major tocopherol in all samples followed by -tocopherol. High proportion of -tocopherol in lettuce compared to other green vegetables has also been found earlier, and it was suggested to reflect early stages of development and low activity of -tocopherol methyltransferase in lettuce 48. Although RB and RCW spectra increased vitamin contents of lettuce plants compared to HPS, none of the LED treatments affected nitrate accumulation.

In conclusion, we found warm-white LEDs as viable light source for lettuce cultivation, since they can provide equal biomass accumulation, nutritional quality and external quality as the conventionally used HPS lamps. The drawback of WW LEDs is their relatively poor energy efficiency compared to HPS lamps which currently limits the utilization of this technology. However, the energy efficiency of WW LEDs is expected to rapidly improve, whereas significant improvements on electrical efficiency of high-intensity discharge lamps are unlikely to occur considering the material limits and the complexity of plasma physics required for these lighting technologies. In addition, no significant improvements in the luminous efficacy for high-intensity discharge lamps have been reported during the last decade 36. Therefore, the energy efficiency of WW LEDs is expected to surpass the performance of current HPS lamps in near future 2,36,49. On the other hand, although RB spectrum reduced biomass accumulation in lettuce when plants were grown in the absence of daylight, it also increased the concentration of vitamins without increasing nitrate levels indicating that LEDs may also have a potential to improve the nutritional quality of the greenhouse crops.

# ACKNOWLEDGEMENTS

We thank Matti Salovaara and Daniel Richterich for technical assistance in greenhouse experiments and M.Sc. Laura Pokela for skillful assistance in folate analyses. The research work was financially supported by the Finnish Funding Agency for Technology and Innovation (Tekes, decision # 40167/09), The Finnish Horticultural Foundation (Puutarhasäätiö), Arrant-Light Oy, Elekno Oy, Elektro-Valo Oy, Greenlux Oy, I-Valo Oy, Oy MTG Meltron Ltd and Osram Oy which together with Aalto University Department of Electronics - Lighting Unit and University of Helsinki, Department of Agricultural Sciences - Horticulture, composed the SSHLighting research project consortium.

# REFERENCES

1     Moe R, Grimstad SO, Gislerod HR. The use of artificial light in year round production of crops in Norway. *Acta Horticulturae* 2006;711:35-42.

2     Pinho P, Jokinen K, Halonen L. Horticultural lighting - present and future challenges. *Lighting Research and Technology* 2012 December 2012;44(4):427-437.

3     Pinho P, Hytönen T, Rantanen M, Elomaa P, Halonen L. Dynamic control of supplemental lighting intensity in a greenhouse environment. *Lighting Research and Technology* 2013 June 01;45(3):295-304.

4     Taiz L, Zeiger E. Plant physiology. 3rd ed. Sunderland, USA: Sinauer Associates Inc.; 2003.

5     Whitelam GC, Halliday KJ editors. Light and plant development. Oxford, UK: Blackwell Publishing Ltd.; 2007.

6     Klein RM. Effects of green light on biological systems. *Biological Reviews of the Cambridge Philosophical Society* 1992 May;67(2):199-284.

7     Kim H, Goins GD, Wheeler RM, Sager JC. Green-light Supplementation for Enhanced Lettuce Growth under Red- and Blue-light-emitting Diodes. *HortScience* 2004 December 01;39(7):1617-1622.

8     Hogewoning SW, Douwstra P, Trouwborst G, van Ieperen W, Harbinson J. An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra. *Journal of Experimental Botany* 2010 March 01;61(5):1267-1276.

9     Lund JB, Blom TJ, Aaslyng JM. End-of-day Lighting with Different Red/Far-red Ratios Using Light-emitting Diodes Affects Plant Growth of Chrysanthemum × morifolium Ramat. ‘Coral Charm’. *HortScience* 2007 December 01;42(7):1609-1611.

10     Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, van Ieperen W, Harbinson J. Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. *Journal of Experimental Botany* 2010 June 01;61(11):3107-3117.

11     Massa GD, Kim H, Wheeler RM, Mitchell CA. Plant Productivity in Response to LED Lighting. *HortScience* 2008 December 1;43(7):1951-1956.

12     Morrow RC. LED Lighting in Horticulture. *HortScience* 2008 December 1;43(7):1947-1950.

13     Brown CS, Schuerger AC, Sager JC. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *Journal of the American Society for Horticultural Science.American Society for Horticultural Science* 1995 Sep;120(5):808-813.

14     Yorio NC, Goins GD, Kagie HR, Wheeler RM, Sager JC. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortScience : A Publication of the American Society for Horticultural Science* 2001 Apr;36(2):380-383.

15     Hoenecke ME, Bula RJ, Tibbitts TW. Importance of 'blue' photon levels for lettuce seedlings grown under red-light-emitting diodes. *HortScience : A Publication of the American Society for Horticultural Science* 1992 May;27(5):427-430.

16     Cummings IG, Foo E, Weller JL, Reid JB, Koutoulis A. Blue and red photoselective shadecloths modify pea height through altered blue irradiance perceived by the cry1 photoreceptor. *The Journal of Horticultural Science and Biotechnology* 2008 01/01;83(5):663-667.

17     Stutte GW, Edney S, Skerritt T. Photoregulation of Bioprotectant Content of Red Leaf Lettuce with Light-emitting Diodes. *HortScience* 2009 February 01;44(1):79-82.

18     Rébeillé F, Ravanel S, Jabrin S, Douce R, Storozhenko S, Van Der Straeten D. Folates in plants: biosynthesis, distribution, and enhancement. *Physiologia Plantarum* 2006;126(3):330-342.

19     Lucock M. Folic Acid: Nutritional Biochemistry, Molecular Biology, and Role in Disease Processes. *Molecular Genetics and Metabolism* 2000 September;71(1–2):121-138.

20     Simonne A, Simonne E, Eitenmiller R, Coker CH. Bitterness and Composition of Lettuce Varieties Grown in the Southeastern United States. *HortTechnology* 2002 October 01;12(4):721-726.

21     Johansson M, Jägerstad M, Frølich W. Folates in lettuce: a pilot study. *Scandinavian Journal of Food & Nutrition* 2007 02/15;51(1):22-30.

22     Lester GE, Makus DJ, Hodges DM. Relationship between Fresh-Packaged Spinach Leaves Exposed to Continuous Light or Dark and Bioactive Contents: Effects of Cultivar, Leaf Size, and Storage Duration. *Journal of Agricultural and Food Chemistry* 2010 03/10;58(5):2980-2987.

23     DellaPenna D, Pogson BJ. Vitamin Synthesis in Plants: Tocopherols and Carotenoids. *Annual Review of Plant Biology* 2006 06/01; 2016/12;57(1):711-738.

24     Lichtenthaler HK. Biosynthesis, accumulation and emission of carotenoids, Î±-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. *Photosynthesis Research* 2007;92(2):163-179.

25     Maeda H, DellaPenna D. Tocopherol functions in photosynthetic organisms. *Current Opinion in Plant Biology* 2007 6;10(3):260-265.

26     Merino L, Darnerud PO, Edberg U, Aman P, Castillo MD. Levels of nitrate in Swedish lettuce and spinach over the past 10 years. *Food Additives and Contaminants* 2006 Dec;23(12):1283-1289.

27     Behr U. Relation between photosynthesis and nitrate content of lettuce cultivars. *Scientia Horticulturae* 1992;49(3):175-179.

28     Blom-Zandstra M, Lampe JEM. The Role of Nitrate in the Osmoregulation of Lettuce (Lactuca sativa L.) Grown at Different Light Intensities. *Journal of Experimental Botany* 1985 July 01;36(7):1043-1052.

29     Samuoliene G, Urbonaviciute A, Duchovskis P, Bliznikas Z, Vitta P, Zukauskas A. Decrease in Nitrate Concentration in Leafy Vegetables Under a Solid-state Illuminator. *HortScience* 2009 December 1;44(7):1857-1860.

30     Kariluoto S, Vahteristo L, Salovaara H, Katina K, Liukkonen K, Piironen V. Effect of Baking Method and Fermentation on Folate Content of Rye and Wheat Breads. *Cereal Chemistry Journal* 2004 01/01; 2016/12;81(1):134-139.

31     Edelmann M, Kariluoto S, Nyström L, Piironen V. Folate in oats and its milling fractions. *Food Chemistry* 2012 12/1;135(3):1938-1947.

32     Caldwell CR, Britz SJ. Effect of supplemental ultraviolet radiation on the carotenoid and chlorophyll composition of green house-grown leaf lettuce (Lactuca sativa L.) cultivars. *Journal of Food Composition and Analysis* 2006 0;19(6–7):637-644.

33     Schwartz H, Ollilainen V, Piironen V, Lampi A. Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. *Journal of Food Composition and Analysis* 2008 3;21(2):152-161.

34     Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem Soc Trans* 1983 10/01;11(5):591.

35     Saure MC. Causes of the tipburn disorder in leaves of vegetables. *Scientia Horticulturae* 1998 8/31;76(3–4):131-147.

36     Pimputkar S, Speck JS, DenBaars SP, Nakamura S. Prospects for LED lighting. *Nat Photon* 2009 print;3(4):180-182.

37     Schuerger AC, Brown CS, Stryjewski EC. Anatomical features of pepper plants (Capsicum annuum L.) grown under red light-emitting diodes supplemented with blue or far-red light. *Annals of Botany* 1997 Mar;79(3):273-282.

38     Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R. Green Light Drives Leaf Photosynthesis More Efficiently than Red Light in Strong White Light: Revisiting the Enigmatic Question of Why Leaves are Green. *Plant and Cell Physiology* 2009 April 1;50(4):684-697.

39     Johkan M, Shoji K, Goto F, Hahida S, Yoshihara T. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in Lactuca sativa. *Environmental and Experimental Botany* 2012 1;75:128-133.

40     Dale JE. The Control of Leaf Expansion. *Annual Review of Plant Physiology and Plant Molecular Biology* 1988 06/01; 2016/12;39(1):267-295.

41     Cosgrove DJ, Green PB. Rapid Suppression of Growth by Blue Light: Biophysical Mechanism of Action. *Plant Physiology* 1981 December 01;68(6):1447-1453.

42     Niizu PY, Rodriguez-Amaya DB. New data on the carotenoid composition of raw salad vegetables. *Journal of Food Composition and Analysis* 2005 12;18(8):739-749.

43     Monge-Rojas R, Campos H. Tocopherol and carotenoid content of foods commonly consumed in Costa Rica. *Journal of Food Composition and Analysis* 2011 3;24(2):202-216.

44     Li Q, Kubota C. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany* 2009 11;67(1):59-64.

45     Vahteristo L, Lehikoinen K, Ollilainen V, Varo P. Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland. *Food Chemistry* 1997 August 1997;59(4):589-597.

46     Konings EJ, Roomans HH, Dorant E, Goldbohm RA, Saris WH, van den Brandt PA. Folate intake of the Dutch population according to newly established liquid chromatography data for foods. *The American Journal of Clinical Nutrition* 2001 April 01;73(4):765-776.

47     De Brouwer V, Zhang G, Storozhenko S, Van Der Straeten D, Lambert WE. pH stability of individual folates during critical sample preparation steps in prevision of the analysis of plant folates. *Phytochemical Analysis* 2007;18(6):496-508.

48     Szymańska R, Kruk J. Tocopherol content and isomers' composition in selected plant species. *Plant Physiology and Biochemistry* 2008 1;46(1):29-33.

49     U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy. Solid-state lighting research and development: multi-year program plan. 2012.

1. \* Corresponding author.

   E-mail address: paulo.pinho@aalto.fi [↑](#footnote-ref-1)