Producing a more valuable crop: improving nutritional value, shelf life, taste, and appearance in microgreens using pre-harvest light treatments

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Inhoud

Definition list

Batch	A group of microgreens with the same harvest day, and sowing date in the same week. They were therefore cultured in the same time period. Batches are numbered from 1 to 8.
PLS regression model	Partial least squares regression model
LCMS	Liquid Chromatography-Mass Spectrometry
LDA	Linear discriminant analysis
PCA	Principal component analysis
One-way ANOVA	One-way analysis of variance
ANCOVA	Analysis of covariance

GROWx

GROWx is a vertical farming company based in Amsterdam. The company has started creating the first fully robotized, zero waste vertical farm to be powered by green electricity. With this system, they want to grow nutritious, affordable, and tasteful produce in and for cities all over the world via farming as a service. The company now successfully sells the produced microgreens via their brand ''Chef's Farm'' to Dutch fine dining restaurants.

Objective of study

This research project is part of a larger research project called GROWx 2.0, which is supported by the European Fund for Regional Development. The GROWx 2.0 project is a collaboration of Wageningen University & Research (WUR), the Amsterdam Metropolitan Solution (AMS), and several other institutions. The project aims to develop an economically viable vertical farming system where cultivation is automated and robotized, and the growth environment is optimized for each species and production phase. GROWx wants to store information on optimal growth environment per plant species in so-called ''plant profiles''. The automated system can use these plant profiles to optimally operate the vertical farm.

This research project focuses on the development of such plant profiles, and thus on the optimization of the growth environment per microgreen and production phase. In particular, on the optimization of the light environment for three microgreens produced by GROWx: Coriander, Mustard Frills Green, and Tatsoi Purple. As GROWx highly values product quality, optimization for quality attributes will be the focus of this research. To efficiently optimize the system, first, more information must be gathered on which quality attributes add value for the consumer, and on potential ways that these attributes can be influenced with light. A literature study was done to fill these knowledge gaps. Next, an experimental study was done to test the potential strategies found in the literature study in the automated vertical farm. The results of this study are written in this research report.

Laymen's summary

Vertical farming, where food is produced in layers on top of each other in a closed system, can play a role in sustainably feeding the cities of the future. GROWx is a vertical farming company that is now developing the first robotized, zero waste vertical farm to be powered with green electricity. On their farm, they produce microgreens, which are young leafy plants. In the vertical farm LED lights supply light to the plants. The intensity and color of light affect many attributes of the plant. The closed environment of a vertical farm offers the potential to tailor the light in a way that very nutritious, tasteful microgreens are produced, with a long shelf life. Using light in such a way is valuable for GROWx as then they would be capable to produce food with higher quality, and thus higher value. At this point, we know little about how light influences the quality of microgreens, especially as every species seems to be affected differently. With this research project, I made a start at researching how GROWx can use light to increase the quality of the microgreens that they produce. Besides that, I worked together with another scientist to create a tool that can speed up future research projects in this area and make them cheaper. In this project, I tested the effect of different light treatments, a few days before the plants were harvested. More specifically, I tested the effect of the intensity of light, and the effect of the ratio between red and blue light used, for Coriander, Mustard Frills Green, and Tatsoi Purple. I measured the effect of these light treatments on various nutritional compounds in the plant, the taste of the plant, the shelf life, and the yield. I found out that light treatments that are given a few days before harvest can affect the quality of the plant, without diminishing the yield. I for instance found that the amount of certain nutritional compounds in Coriander is increased by red light, that blue light creates sweeter Tatsoi Purple microgreens, and that in Mustard Frills Green light with a high light intensity results in microgreens with a longer shelf life. It is clear though that we have a long way to go to fully understand the effect of light on the different microgreens. We, therefore, developed a model that can predict the compounds in a plant with visual analysis, which could save time and lab costs. Unfortunately, the models that were developed were not useful. It remains interesting to look into the development of models like this, but a different method must be sought. To summarize, this research highlighted the potential of using light to improve microgreen quality offers and studied how to continue exploring this route. With that, this project helps GROWx to produce a vertical farming system in which added value can be created.

Abstract

Vertical farming systems, as they do not receive outside inputs, provide opportunities to optimize the environment for the species cultured. Recently, pre-harvest light treatments to improve crop quality, have gained interest. Such treatments have a lot of potential, as they could increase crop quality efficiently and cost-effectively. In this research project, the use of pre-harvest treatments to improve microgreen quality was investigated for the vertical farming company GROWx. In the study, the effect of pre-harvest (5 days) light intensity (140 – 640 µmol m^{-2} s^{-1}) and spectrum (10% - 50% blue) treatments were researched in Coriander, Mustard Frills Green, and Tatsoi Purple microgreens. It was found that the concentration of metabolites, the taste, and the shelf life of the microgreens was influenced by the light treatments, while yield was not. However, environmental variation was present during the experiment which could have influenced the findings. Not all aspects of each of the attributes were influenced. For nutritional value, the concentration of specific phenols, carotenoids, and glucosinolates was affected by the treatments, while the concentration of chlorophyll, ascorbic acid, α-tocopherol, and sugars remained unaffected. Knowledge of the effects of light treatments on specific compounds was generated. One of the findings for instance was that low light intensity decreased carotenoid content in Coriander. Regarding taste, the bitterness, sweetness, firmness, and color of the greens were changed by the pre-harvest treatments. Other attributes like overall liking, smell, and specific aromas were not. No singular treatment stood out to be most effective to increase overall quality, effects were specific for each attribute. Lastly, all effects on quality attributes were found to be species-specific. This research project showed the potential that pre-harvest treatments offer to specifically increase certain attributes in microgreens, but it also highlighted the need for additional research projects. In addition to the experimental study, this research project aimed to develop PLS regression models that predict the concentration of certain plant compounds with VIS-NIR spectrometry, to support future studies on the optimal environment. Unfortunately, the models that were developed were not useful due to low accuracy. It remains interesting to put efforts towards the development of such models.

1. Introduction

The global population is increasing rapidly, and with it is the need for food. In addition, the number of people living in cities is increasing, which further complicates the challenge to produce enough food for everyone and for it to be accessible. Urban agriculture with closed vertical farming systems can be part of the solution and could supply cities with fresh agricultural products (Graamans et al., 2018). Additionally, when these farms use their closed systems to optimize the growth environment for the crops cultivated, added crop value can be created and energy input can be minimized (Kozai et al., 2015). Light spectrum and light intensity are important parts of the growth environment and can be used for this, as they have an impact on various quality attributes and yield. Recently, pre-harvest light treatments, which usually take place three to five days before harvest, have gained attention as a potential method to stir crop quality and yield, in a time-efficient and cost-effective manner. In earlier research, such treatments have shown to be effective in altering the nutritional value and yield of crops. As an example: Samuoliene and colleagues (2012) found that the ascorbic acid content increased up to 79.5% when supplemental red LED lights were used three days before harvest.

In vertical farming systems mostly, small leafy greens are produced. These small crops can grow efficiently in stacked horizontal systems because of their size and have a relatively short production period which increases the profit of the farm (Beacham et al., 2019). Microgreens, an emerging specialty crop, have these qualities and can therefore be efficiently produced in vertical farms (Tavan et al., 2021). Microgreens are immature greens with a growing period in between that of cresses and baby greens. They are known for their sharp flavors, vivid colors, and high nutritional value. Their high nutritional value is caused by the high concentration of secondary metabolites in these plants, which have antioxidant properties. They are a source of carotenoids, ascorbic acid, α-tocopherols, phenolic compounds, and macro- and microminerals. Because of it, they can enhance the human diet and prevent a range of common diseases (Xiao et al., 2015). Some, therefore, refer to microgreens as a novel ''functional food'' (Choe et al., 2018). GROWx, an innovative vertical farming company located in Amsterdam, is cultivating about forty microgreen varieties and is aiming to create the first fully optimized and automated vertical farm. Quality of the crops is important for the company, as produce is sold to local fine-dining restaurants. The vertical farming system with its ability to control the light environment to a great extent could enable GROWx to produce microgreens with higher quality. Especially the use of pre-harvest treatments for this are interesting, as light treatments at the end of the growth period have proven to be impactful, and are relatively easy to implement (Nicole et al., 2019; Samuoliene et al., 2012; Steindal et al., 2016).

Crop quality is intertwined with market value and can be defined by a combination of many attributes. For an increasing amount of consumers, nutritional value is one of these attributes (Xiao et al., 2015). Other important factors are appearance, shelf life, and taste (Michell et al., 2020; Nicole et al., 2019). All can be influenced by light. To illustrate: spectra with higher blue light percentages in a range of 5%-30% resulted in darker cotyledons for red-leafed mustard and less pure-green cotyledons in cabbage and arugula (Ying et al., 2020). Furthermore, a pre-harvest project found that the shelf life of baby spinach and rocket was increased by several days when light with a high blue light content (35%) was used (Nicole et al., 2019). Often, microgreens have a shelf life of just a few days. An increase in shelf life as described by Nicole et al. (2019) can therefore already be quite meaningful for companies and consumers (E. Kolmer, *personal communications*, 11-5-2021). Earlier research showed that microgreen taste can be influenced by both light spectrum and intensity (Litvin et al., 2020; Nicole et al., 2019).

More specifically, a higher light intensity resulted in a more intense flavor in the study of Nicole et al. (2019), and Litvin et al. (2020) showed that blue LED light could increase the concentration of flavor-related compounds. Taste is however a difficult quality attribute to improve, as it is influenced by many different compounds and the perception of the attribute is influenced by factors like memory and learning (Mouritsen, 2015).

Interestingly, appearance, shelf life, taste, and nutritional value are all related to different compounds and their concentrations in the plant. Microgreen color is linked to the pigments present among which: chlorophyll, anthocyanin, and carotenoids. Furthermore, visual quality, an important aspect of shelf life, was found to be correlated to initial dry weight, ascorbic acid, individual soluble sugars, and total soluble sugars (Min et al., 2021; Mir et al., 2017). Lastly, a product gets a certain taste by a combination of different compounds that make up a flavor profile. Phenols, a group of compounds with an aromatic benzene ring and one or more hydroxyl groups, play a role in this flavor profile (Zhang et al., 2020). In recent research, it was found that the compounds are tightly linked to astringency, sourness, and bitterness characteristics (Caracciolo et al., 2020). The compounds that play a role in defining the quality attributes mentioned are also compounds that have nutritional benefits. Ascorbic acid, phenols, carotenoids, and chlorophylls all are bioactive compounds with a high antioxidant capacity and play a role in modulating inflammation and immune system responses at the cellular level (Zhang et al., 2020). Although little is known about the biochemical pathways behind the production of these compounds, it is known that light plays a role in them.

Studies showed that ascorbic acid can be increased in tatsoi and red pak choi when a lower light intensity is used (Samuoliene et al., 2013) and that it can be increased with more red or more blue light depending on the species and study (Meas et al., 2020; Samuoliene et al., 2012). Phenols on the other hand were found to increase with light intensity, although for some species, among which mustard, no increase was found (Samuoliene et al., 2013). Similar results were obtained when a high light intensity pre-harvest treatment was tested where alfalfa, broccoli, and radish sprouts were given 700 µmol m^{-2} s^{-1} for one day. The treatment did however decrease fresh weight (Oh & Rajashekar, 2009). Light spectrum also influences the concentration of phenols. Phenols are mostly increased by blue light, which is likely caused by the higher photooxidative stress that blue light causes due to shorter wavelengths, and the role that the compounds play to protect the plant from photo-induced damage (Zhang et al., 2020). Red light also plays a role in the accumulation of phenols, as it was found that a combination of red and blue light increases the synthesis of phenolic compounds more than monochromatic red or blue light. This is likely caused by the fact that red and blue light are both absorbed by chlorophyll and together can modulate different pathways for syntheses (Alrifai et al., 2019). Here again, species responded differently to light intensity and spectrum. Lobiuc et al. (2017) showed that in some cases this might have something to do with the pigments in the plant, more specifically with the different regulatory mechanisms of the Phenylalanine ammonia-lyase enzyme in red and green tissues, which plays an important role in the synthesis of phenols (Lobiuc et al., 2017). Carotenoids and chlorophylls are also mainly increased by blue light, and higher light intensity (Alrifai et al., 2019; Lobiuc et al., 2017). Although, again, in practice often a combination of red and blue light seems optimal for improving plant health and crop quality (Zhang et al., 2020). As shown, species are affected differently, and thus have another optimal light environment. A lot is still unknown about why species are affected differently, as the biochemical pathways that play a role are still unclear. Still, it is interesting to focus on the effect of light on these compounds and higher-level quality attributes, in efforts to improve the quality attributes of crops. However, determining the optimal light environment for each species separately is very costly and time-consuming. It is therefore also interesting to look for ways to speed up this process. Spectrometry models that can predict the relative abundance of

nutritional compounds could be part of the solution and would fit the aim of GROWx to create a fully automated and optimized vertical farm. In the past, spectrometry has proven to be a useful tool for evaluating internal quality attributes. In 2010 a research project showed that visible and near-infrared spectroscopic techniques (VIS/NIR) could be used to develop a model that predicts soluble solids content and acidity (pH) (Moghimi et al., 2010). Another research project was able to use NIR spectrometry to predict glucobrassicin concentrations in cabbage and brussel sprout leaf tissue, which is an important nutritional compound for cancer prevention (Renner & Fritz, 2020).

In this research project, a study is conducted that contributes to the optimization of the light environment at GROWx, as part of the GROWx 2.0 project. In the GROWx 2.0 project, GROWx, Wageningen University and Research (WUR), and the Amsterdam Institute for Advanced Metropolitan Solutions (AMS) collaborate to develop, optimize, and automate the companies vertical farming system. The effect of pre-harvest light on microgreen quality was studied, to efficiently improve crop quality and market value of three different microgreen species. The research question of the study is defined as: ''How are the nutritional value, shelf life, and taste of Coriander, Mustard Frills Green, and Tatsoi Purple affected by pre-harvest treatments?'' The effect of both lower and higher light intensities, and spectra with different percentages of blue light, were tested and compared to each other and normal conditions. It is hypothesized that all quality attributes are influenced by the pre-harvest light treatments and that especially treatments with higher light intensity or more blue light are effective to increase quality. This hypothesis is based on some pre-harvest studies, and several research projects on the effect of light treatments during the whole growth period (Alrifai et al., 2019; Choe et al., 2018; Gómez & Jiménez, 2020; Oh & Rajashekar, 2009; Samuoliene et al., 2013; Zhang et al., 2020). However, it is also expected that some compounds are increased with lower light intensity and red light treatments (Meas et al., 2020; Samuoliene et al., 2012; Samuoliene et al., 2013). It is also thought that light treatments could increase crop value on the one hand, but decrease another aspect of the crop, resulting in partial optimization. For instance, it is expected that high light intensity and blue light treatments could decrease fresh weight, which negatively affects farm income, in addition to increasing crop quality, which creates value (Gómez & Jiménez, 2020; Ying et al., 2020). Furthermore, it is expected that the species will react differently to the light treatments, including showing not to be affected, as such effects were shown in various studies (Jung et al., 2021; Zhang et al., 2020). Besides this cultivation study, effort has been put towards the development of PLS regression models that can predict the concentrations of plant compounds with VIS-NIR spectrometry data, to support the many studies still needed to fully optimize the growth environment and GROWx for crop quality and efficiency.

2. Methods

2.1 Plant material and growth conditions

Coriander (Coriander Splits (micro), *Coriander sativum*), Mustard Frills Green (Oriental Mustard Golden Streaks, *Brassica juncea*), and Tatsoi Purple (Shiny Sun, *Brassica rapa narinosa*) seeds were retrieved from CN Seeds (Stock 51552), Tozer Seeds (Stock 10454165), and Uniseeds (Stock 18090420) respectively. The Coriander seeds were washed for 24h with diluted bleach (<5%, 2 ml/L). For this, seeds were put in a bucket filled with water and bleach, in which the dilution was constantly moving. Afterward, the seeds were taken out and air-dried, before being used. The Mustard Frills Green and Tatsoi Purple seeds were used directly. The seeds were sown on cellulose tissue (InseroTech) which was placed in long vertical metal gutters. Per gutter, 2760, 5379, and 6538 seeds were used for

Coriander, Mustard Frills green ant Tatsoi Purple respectively. The seeds were then germinated in 3-5 days in a dark germination cell with a temperature between 25,5 - 28 °Celsius and a relative humidity (RH) of 80-90%. Next, the gutters were placed in the automated vertical farming cell. An overview of the cell can be found in Appendix A. The target day temperature in this cell is 24 °Celsius with an RH of 70% and the target night temperature is 20 °Celsius with an RH of 70%. An overview of the realized mean temperature and humidity in the cell, which differed quite a lot from the target values, can be found in Appendix B. In the growing cell, the gutters were first placed in the pre-growth layer, where they stayed for 7 days. LED light with a light intensity of 150 µmol $m^{-2}s^{-1}$, and a spectrum of R70:G10:B20 was present here, and plants received no nutrients. Next plants were transferred to the growth layer where they remained for 7 days. Here a light intensity of 240 µmol m^{-2} s^{-1} LED light was used with a spectrum of R70:G10:B20 and plants were given nutrients (Appendix C). Lastly, plants were brought to the final layer, the pre-harvest layer. The plants were kept here for five days, after which they were harvested. During this last phase, microgreens were exposed to a variety of light treatments. Watering was done three times a day by robots that were positioned on each layer in the vertical farm. The amount of water that was given to a gutter depended on the weight of the gutter versus the weight goal of that gutter, which differed for the pre-growth, production, pre-harvest layer.

During cultivation, LED lamps (Production module 3.0, Signify) were used. For standard production, a light spectrum with an R70:G10:B20 ratio is used with a light intensity of 240 µmol m^{-2} s^{-1}. In this experiment, the standard spectrum and light intensity are used during production up until the pre-harvest treatments. The last 30 minutes before the lamps are turned off, the plants received 40 - 50 µmol m^{-2} s^{-1} far-red light while the lamps faded out. Plants are grown under 13h-light/11h-dark conditions during the pre-growth and growth phase.

2.2 Experimental design

This study can be divided into the pilot, the main experiment, and the taste experiment. All together six experimental and one control treatment were tested for five days before harvest in this study (Table 1). First, a pilot study was done to test and validate the experimental methods and to create a broad idea about the effects of the treatments on the microgreens. In this pilot, the 440 µmol m^{-2} s^{-1}, R60:B40, R90:B10, and control treatment were tested (Tab. 1). One gutter with microgreens grown on substrate of 10 x 230 cm is considered a unit for the pilot and main experiment. During the pilot, four replicas per treatment were tested, divided over two time periods. Gutters that received treatment in the same week are considered to be from the same batch. In the main experiment six different experimental treatments and the control treatment were tested (Tab. 1). Three of these experimental treatments and the control treatment were already tested in the pilot, as mentioned. Generally speaking, for Mustard Frills Green and Tatsoi Purple here again four replicas per treatment were tested, divided into different batches. The exact number of replicas per treatment differs for some treatments due to practical implications. For Coriander, the number of gutters varied between six and nine for the control, the 440 µmol m^{-2} s^{-1}, R60:B40, and R90:B10 treatment. The development of the NIR model required a higher number of samples to be analyzed. For some gutters therefore three pieces of substrates were tested for the parameters needed, resulting in n=19-20. The experimental unit remained defined as a gutter for all other analyses that were done. For the 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} $s^{-1,}$ and R50:B50 treatment four replicas were used (Table 1). No changes in experimental methods were made in the main experiment compared to the pilot, except for shelf life. All data from the pilot and main experiment, apart from shelf life, are therefore analyzed together. Lastly, a taste experiment was done. For the taste experiment, a box of harvested microgreens that is served to a participant is

considered an experimental unit. Two gutters per treatment per species were cultivated for the taste testing itself and two more for each species were cultivated for the training.

Tab. 1: Pre-harvest treatments used in this study. Treatments were implemented 5 days before harvest.

Treatment	***Red (%)***	***Green (%)***	***Blue (%)***	***Intensity (µmol/m2/s)***	***Photoperiod***
Control	*70*	*10*	*20*	*240*	*13h*
140 µmol/m2/s	*70*	*10*	*20*	*140*	*11h*
440 µmol/m2/s	*70*	*10*	*20*	*440*	*11h*
640 µmol/m2/s	*70*	*10*	*20*	*640*	*11h*
R90:B10	*90*	*0*	*10*	*240*	*13h*
R60:B40	*60*	*0*	*40*	*240*	*13h*
R50:B50	*50*	*0*	*50*	*240*	*13h*

2.3 Biochemical determination

A piece of substrate with about 25 grams of microgreens was cut from the total substrate on the day of harvest and was delivered to Wageningen University & Research for analysis. After spectrometric analysis (2.7. development model), the microgreens were harvested and frozen with liquid nitrogen. Next, the frozen samples were ground into a powder and stored at -80 °Celsius, until further analysis.

During the pilot, nutritional value of Coriander, Mustard Frills Green, and Tatsoi Purple microgreens was studied with analysis of the concentration of phenols, carotenoids, glucosinolates, chlorophyll a and b, glucose, fructose, sucrose, ascorbic acid, and α-tocopherol. Carotenoids, ascorbic acid, and chlorophyll a and b were analyzed with HPLC-PDA (Zafeiropoulou et al., 2020). Ascorbic acid was further quantified with a calibration curve (Alós et al., 2015). Tocopherol was analyzed with HPLC and quantified with fluorescence detection (Wu et al., 2006). The glucosinolates and phenols were analyzed with LCMS (Nakabayashi & Saito, 2013). Lastly, sugars were analyzed with a Dionex (Guignard et al., 2005). In the main experiment, a deeper insight into the effect of the treatments on metabolites was generated, by specific analysis of carotenoids, chlorophylls, and tocopherols in Coriander with a higher number of repetitions. These compounds were again analyzed with HPLC-PDA. All metabolites were analyzed in the lab by the research group ''Breeding for Quality'' of Wageningen University and Research. Next to analysis of individual compounds, principal component analysis (PCA) based on correlation was performed for all log-transformed metabolites (treatment and effect of batch), and for all log-transformed metabolites which concentration differed significantly between treatments. This was done using Past.4.03.exe.

2.4 Shelf life

During the pilot, shelf life was measured visually using a visual assessment scale from 1 to 5, as was done in the study of Berba & Uchanski (2012). Furthermore, weight loss was measured, as this is an indicator of quality loss. Microgreens were stored at 4 °Celsius. The data of this experiment is not shown, as the research method was changed in the main experiment.

During the main experiment, shelf life was only analyzed by monitoring weight loss. Measurements took place twice a week. Boxes of microgreens remained closed during the whole experiment. During the main experiment boxes with microgreens were kept in a storage unit with a constant temperature of 10 °Celsius. In both experiments, weight loss was measured according to equation 1.

$$Weight\ loss\ (t) = 1 - \frac{weight(t,t)}{weight(t,0)} * 100\% \qquad (1)$$

2.5 Taste and appearance

A taste experiment was done for the 440 µmol m^{-2} s^{-1}, R60:B40, R90:B10, and control treatment, in which also appearance was scored. The taste was analyzed by several chefs and employees of GROWx. The participants in this study were also asked to score appearance. A method based on the Flash profile method (Bredie et al., 2017), which is a descriptive method, was developed for the analysis. First, participants were invited to participate in a tasting session of all three microgreen species. During this first session, participants were asked to individually analyze, smell, and taste each species and write down all the different attributes (e.g. aromas) that came into mind. Afterward, all attributes were discussed. In the end, a clear list of important attributes per species was formed, and unanimity was reached under the participants on how each attribute must be analyzed. This list of attributes was used to develop a scoresheet where participants could score the intensity of the attribute on a line scale by placing a vertical mark. In Appendix D one of the scoresheets that was used can be found.

The second part of the taste experiment consisted of three tasting sessions. Each week one species was tested. For this, participants received four boxes of microgreens, corresponding to the four treatments. Each box was numbered with a different three-digit code, to ensure that participants were not able to link the treatments to the microgreens. Next to this, they received some neutralizing crackers, a scoresheet, and a manual with information on the test procedure. Participants were asked to first score appearance, continue with smell, and end with scoring the taste. It was allowed to identify the microgreens of different treatments as identical when the participant could not differentiate the samples based on the attribute. When the participant was not able to differentiate microgreens at all for a certain attribute no data was generated, with the result that the data could not be used for analysis. After scoring, the samples were ranked per attribute according to the vertical marks that were placed on the line scale. For practical reasons, the number of participants varied per session. In the taste experiment for coriander and mustard, six people participated, while for tatsoi four people took part.

2.6 Yield attribute evaluation

Microgreens were harvested by cutting the microgreens from the substrate. After harvesting, the fresh weight of the yield per gutter was measured. In the instance that part of the gutter died during cultivation, the remaining yield was measured. For data analysis, the dead part of the gutter was compensated for relative to the length of substrate with dead microgreens, according to the measured fresh weight per cm of the healthy microgreens of that gutter. Next, per gutter, about 20 grams of microgreens (fresh weight) were dried in an oven for 24-48 hours at 105 °Celsius. After this period, the dry weight was analyzed and compared to the initial fresh weight. This information was used to calculate the dry weight % (Equation 2). In addition to that, the hypocotyl length of five microgreens per gutter was measured. Therefore, in this project yield is assessed in a broad way. For statistical analysis, the average hypocotyl length per gutter was used.

$$Dry\ weight\ \% = \frac{Dry\ weight}{Fresh\ weight} * 100\% \qquad (2)$$

2.7 Development model

The pieces of substrate with microgreens that were biochemically analyzed were first brought to a spectral imaging set-up at Wageningen University & Research. The set-up consists of two spectral

cameras: a Specim FX10, covering the Vis-NIR spectral range (400-1000 nm), and a Specim FX17, covering the short-wave infrared spectral range (SWIR, 900-1700 nm). Both cameras are attached to an x-y stage, which makes it possible to scan the pieces of substrate, thereby recording full spectral images in the Vis-NIR and SWIR. The images from both cameras were segmented, to be able to selectively extract spectral data from the microgreens. The resulting average plant spectra were then used for the development of a partial least squares (PLS) regression model. It was found early on that the data from the SWIR camera had less predictive power than the data from the Vis-NIR camera. For that reason, only the Vis-NIR data is discussed in the remainder of this report. The development of the model was done by a scientist of the Greenhouse Horticulture Business Unit of Wageningen Research.

2.8 Statistical analysis

Statistical analysis was used to look for significant differences in the different parameters measured, under different light conditions, and other environmental factors (effect of batch). Statistical analysis was done in IBM SPSS (24th edition). During this experiment, a 95% confidence interval was used. In this report, two-sided p-values are reported.

For fresh weight, dry weight, and hypocotyl length, normality was tested with a Q-Q plot, and homogeneity of variances was tested with Levene's test. When normality could not be assumed the Kruskal-Wallis statistical test was performed. When significance was found, the data was further tested with Man-Whitney statistical tests. When normality could be assumed, but no homogeneity of variances was present, a Brown-Forsythe test was done to test for significance. A Games-Howell test was done for further analysis when significance was found. In all other cases, One-way ANOVA tests were done, with LSD posthoc when significance was found. The taste and the nutritional compounds were analyzed similarly. For taste, however, data was first ranked. For the nutritional compounds, metabolites with missing values were taken out before data analysis. After this, data were log-transformed and statistically analyzed with ANOVA and PCA. Lastly, shelf life was statistically analyzed with an ANCOVA model with treatment as factor and days after harvest as covariate. Statistical differences between treatments were then analyzed by looking at the difference in parameter estimates per between treatments for days*treatments (slope).

3. Results

3.1 Biochemical analysis pilot study

This project aims to increases the quality of microgreens with pre-harvest light treatments. Nutritional value is an important part of the quality. In order to determine the effect of the pre-harvest light treatments on nutritional value of the microgreens, the metabolites in the plant were analyzed with LCMS and HPLC-PDA. In the pilot, a vast amount of data was generated, especially for carotenoids, phenols, and glucosinolates. In total eleven different carotenoids, 25 different glucosinolates, and >1000 different phenols were found. However, in some samples no data were obtained for many of these compounds, resulting in missing values. In data analysis, these compounds were excluded. In total, a few hundred compounds per species remained and were further studied. Besides these compounds, chlorophyll a and b, glucose, fructose, sucrose, α-tocopherol, and ascorbic acid were studied during the pilot. The biochemical analysis showed that the concentration of some carotenoids, phenols, and glucosinolates was affected by the light treatments, showing potential to steer nutritional quality with pre-harvest light treatments. The concentration of ascorbic acid, α-tocopherol, chlorophyll a and b, sugars and other carotenoids, phenols, and glucosinolates were unaffected. In Coriander, for seventeen metabolites a significant difference in concentration was found when microgreens of the

control, R90:B10, R60:B40, and 440 µmol m^{-2} s^{-1} treatment were compared. In Mustard Frills Green and Tatsoi Purple, a significant effect was found for eight and three metabolites, respectively. This indicates that coriander microgreens are relatively subjective to the treatments, while the metabolite content of Tatsoi Purple remained relatively unaffected. To better understand the effects of the pre-harvest treatments on the nutritional value of each of the species, the next section zooms in on the effect of the treatments on individual metabolites and overall metabolite composition, starting with Coriander.

Looking at the effect of treatment on individual metabolites it becomes clear that most compounds are not increased by a specific treatment but are increased by multiple pre-harvest treatments (Fig. 1). Still, for Coriander, most increases in metabolites were caused by the treatment with more red or more blue light. Especially the R90:B10 treatment seems to be effective to increase metabolite abundance. Next, to get an idea of the effect of pre-harvest treatments on overall metabolite composition, the seventeen metabolites for which a significant effect of treatment was analyzed with PCA (Fig. 2). As this study aims to identify methods to improve overall nutritional value in microgreens, looking at the effects on a larger scale is important. During the experiment, water gift, temperature, and humidity in the cell varied (Appendix B). To get an idea of the impact of these variations, the effect of batch, which represents the period in which the plants were cultured, was analyzed with PCA as well (Appendix E).

In the PCA for the metabolites that were significantly affected by treatment, PC1 and PC2 both represent part of the variance (37,2% and 24,2%). The total variance that can be explained by these two principal components is thus 61,4%, which is a relatively medium amount (Fig. 2 – B & C). As it was found that treatments influence the metabolite concentration of these metabolites, the effect of treatments is thought to be represented by one of these PCs. The two PCs that were considered in the analysis, represent relatively equal amounts of variance in the data. This indicates that next to treatment, another factor also largely affects the metabolite content within the species. This factor is thought to present the effect of batch (Appendix E). It is thought that in Coriander, batch causes more variation in metabolite concentration than treatments. PC1 is therefore considered to represent variation caused by batch and PC2 is thought to represent variation caused by treatment. This will be further explained at the end of section 3.1.

Looking more into depth at the variation in metabolite composition that is caused by the treatments, it becomes clear that the individual metabolites react differently to the treatments. The correlation biplot shows that, even within the phenols group, the metabolites are affected differently by the principal components (Fig 2 – A). To get insight into which metabolites were affected most severely by the principle components for each PC the important contributors are highlighted (Fig. 2 – B). For PC1 phenol 25, 42, 1707, 127, 161, 1679, 1012, 33 cause most variation, and are thought to be most influenced by batch. For PC2 phenol 127, 657, 262, 239, 679, carotenoid 7, carotenoid 4 are the largest contributors to variation, indicating that these metabolites are most influenced by treatment. At this point, it is still unclear which specific compounds the different phenols and carotenoids represent.

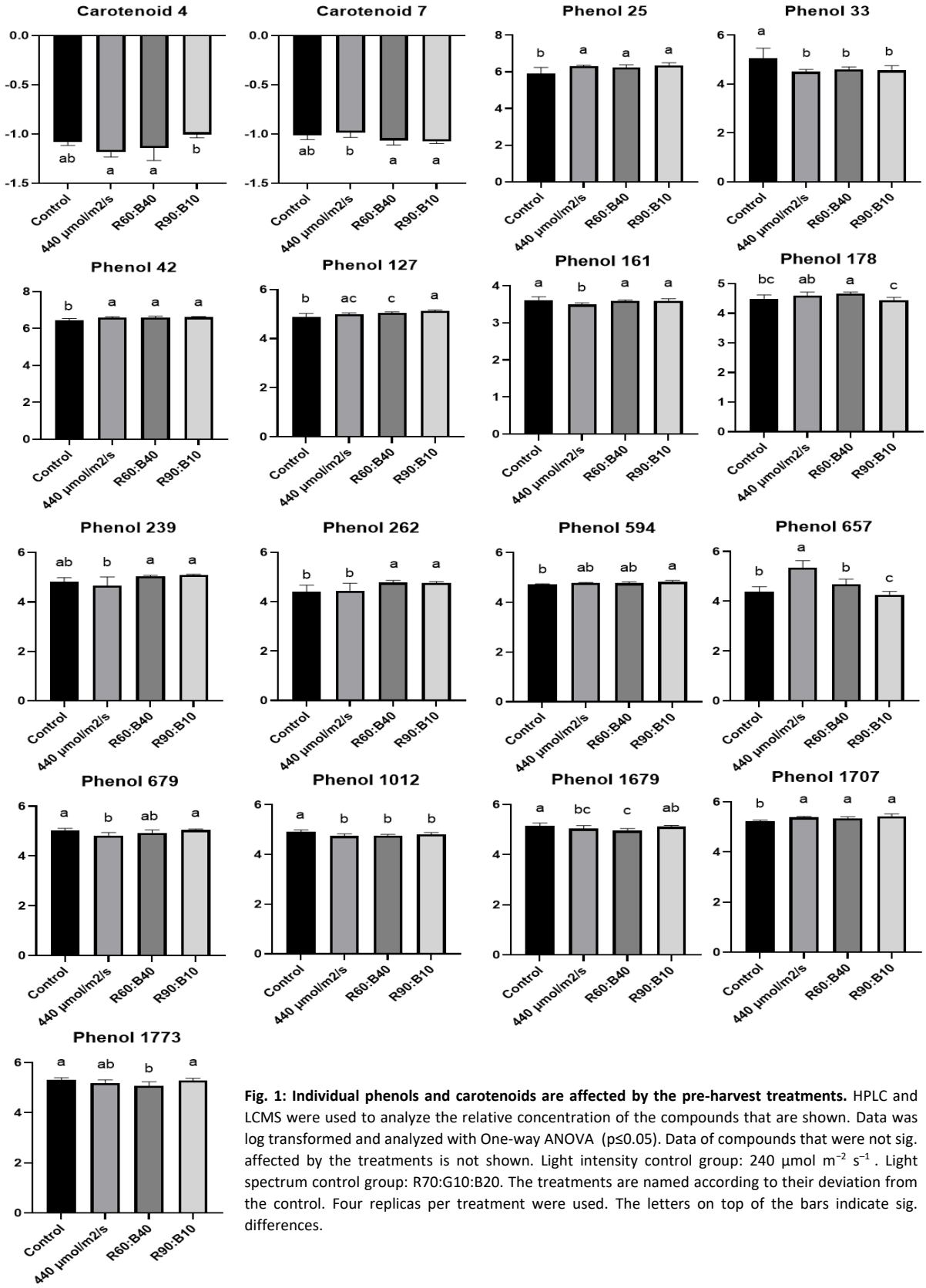
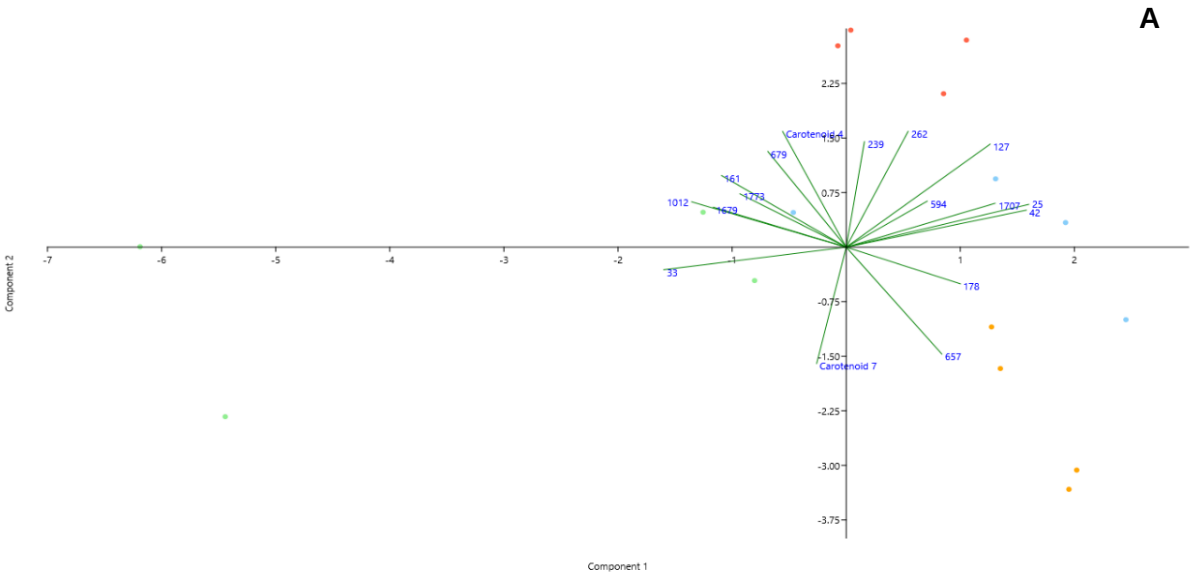


Fig. 1: Individual phenols and carotenoids are affected by the pre-harvest treatments. HPLC and LCMS were used to analyze the relative concentration of the compounds that are shown. Data was log transformed and analyzed with One-way ANOVA ($p \leq 0.05$). Data of compounds that were not sig. affected by the treatments is not shown. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. The treatments are named according to their deviation from the control. Four replicas per treatment were used. The letters on top of the bars indicate sig. differences.



	PC 1	PC 2
Phenol 25	**0.36195**	0.13316
Phenol 42	**0.35715**	0.11502
Phenol 1707	**0.29517**	0.13634
Phenol 127	**0.28539**	**0.32028**
Phenol 178	0.2264	-0.11398
Phenol 657	0.18961	**-0.33252**
Phenol 594	0.16062	0.14296
Phenol 262	0.12285	**0.36021**
Phenol 239	0.035994	**0.32816**
Carotenoid 7	-0.05862	**-0.36194**
Carotenoid 4	-0.12611	**0.35991**
Phenol 679	-0.15553	**0.29766**
Phenol 1773	-0.21089	0.16583
Phenol 161	**-0.24721**	0.22268
Phenol 1679	**-0.26346**	0.12416
Phenol 1012	**-0.30663**	0.14104
Phenol 33	**-0.36183**	-0.07006

Fig. 2: A pattern based on treatments is found when the phenols and carotenoids that were sig. affected (p≤0.05) by pre-harvest treatments (5 days) are analyzed with PCA in Coriander. A: scatterplot with biplot (correlation). **B**: loadingsplot. **C**: loadings (filtered on values PC1 high to low). The different color dots in fig - A represent the treatments. Green: control treatment (R70:G10:B20 – 220 µmol m^{-2} s^{-1}), Orange: 440 µmol m^{-2} s^{-1} treatment, Red: R90:B10 treatment Blue: R60:B40 treatment. The treatments are named according to their deviation from the control. Log transformed HPLC-PDA and LCMS data was used in the PCA. Statistical testing was done with One-way ANOVA. Four replicas per treatment are used.

Next to Coriander, the effect of pre-harvest treatments on nutritional value was analyzed in Mustard Frills Green. Again, first, the effect of the treatments on individual metabolites was analyzed. In Mustard Frills Green eight metabolites differed significantly in concentration when microgreens of the control, R90:B10, R60:B40, and 440 µmol m^{-2} s^{-1} treatment were analyzed (Fig. 3). No clear conclusion can be drawn on which treatment was most successful in increasing the concentration of the individual metabolites most, as no clear pattern is observed. For the glucosinolate compounds though, a specific response seems to be present, as both were increased by the control treatment (Fig. 3).

To get a better overview of the overall effect of the pre-harvest treatments on the affected metabolites in Mustard Frills Green, the compounds were analyzed with PCA (Fig. 4). PC 1 was found to explain about half of the variance in the samples (45%), and PC2 was found to explain 28,8%. As PC3 was found to explain a lot less of the variance, only PC1 and PC2 were considered during further analysis (Fig. 4 - C). The total variance that can be explained by the two principal components is 73,8%, which is

relatively high. For mustard, it was also found that batch has a larger effect on the metabolites compared to treatment (Appendix E). It is therefore thought that PC1 represents variation caused by batch and PC2 represents variation caused by treatment. Still, the scatterplot of the PCA in which the effect of treatments can be analyzed, shows that plotting the data against the two PCs results in clear separation of treatment groups (Fig 4 – A). Therefore, when these metabolites are analyzed an overall effect of treatment becomes visible.

To get more knowledge on how the principle components are contributed to by the different compounds, the important metabolites for each PC are highlighted (Fig. 4 – B). It is shown that phenol 113, 70, and 674 contribute most to the variance presented by PC1, which likely represents variation caused by batch. For PC2 these are: 4-methoxy-3-indolylmethyl-glucosinolate, 1-methoxy-3-indolylmethyl-glucosinolate, carotenoid 6 and phenol 99 (Fig. 4 – B). These compounds are likely the largest contributors to the treatment effect. It is still unclear which compound phenol 99 represents. For carotenoid 6 it is thought that the compound might be cryptotoxin. This has however not been validated.

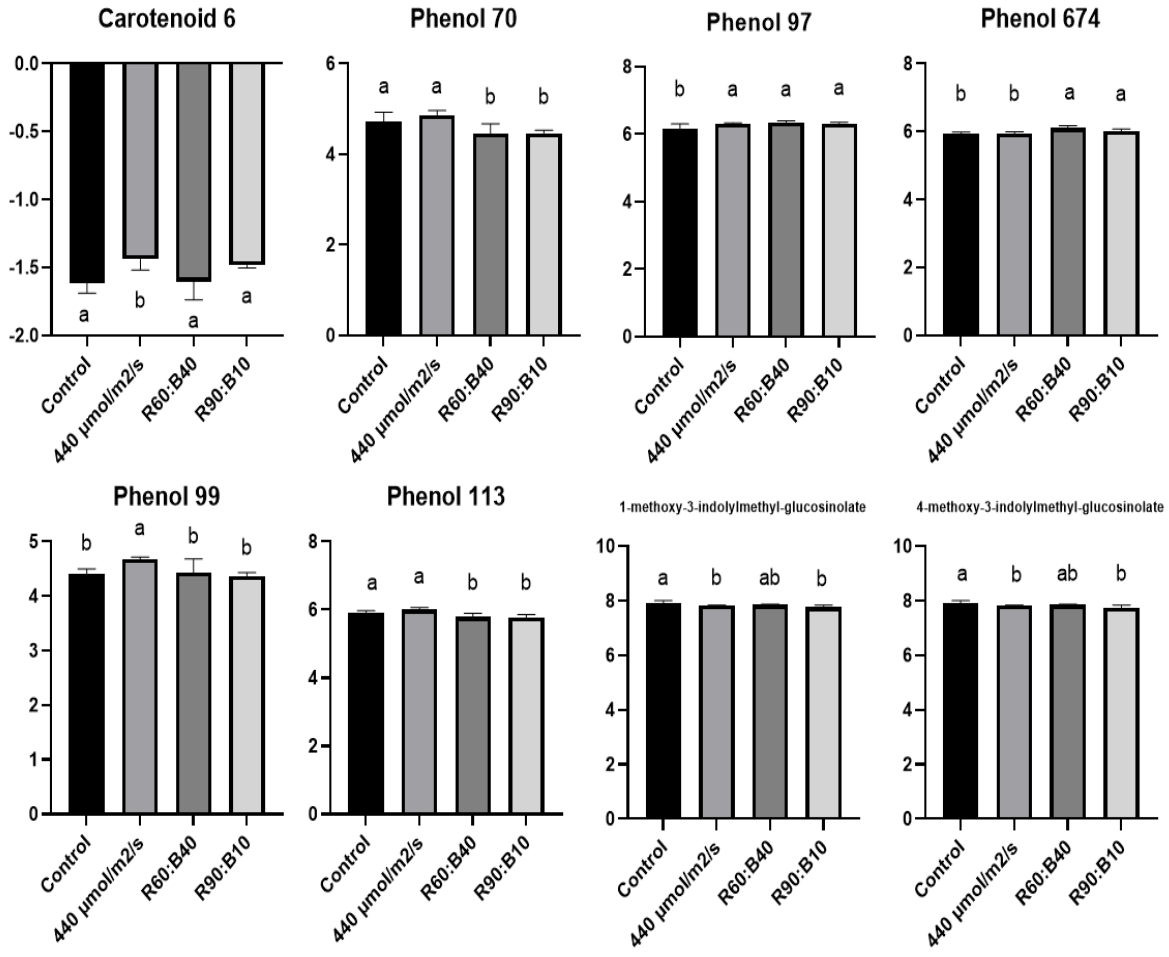
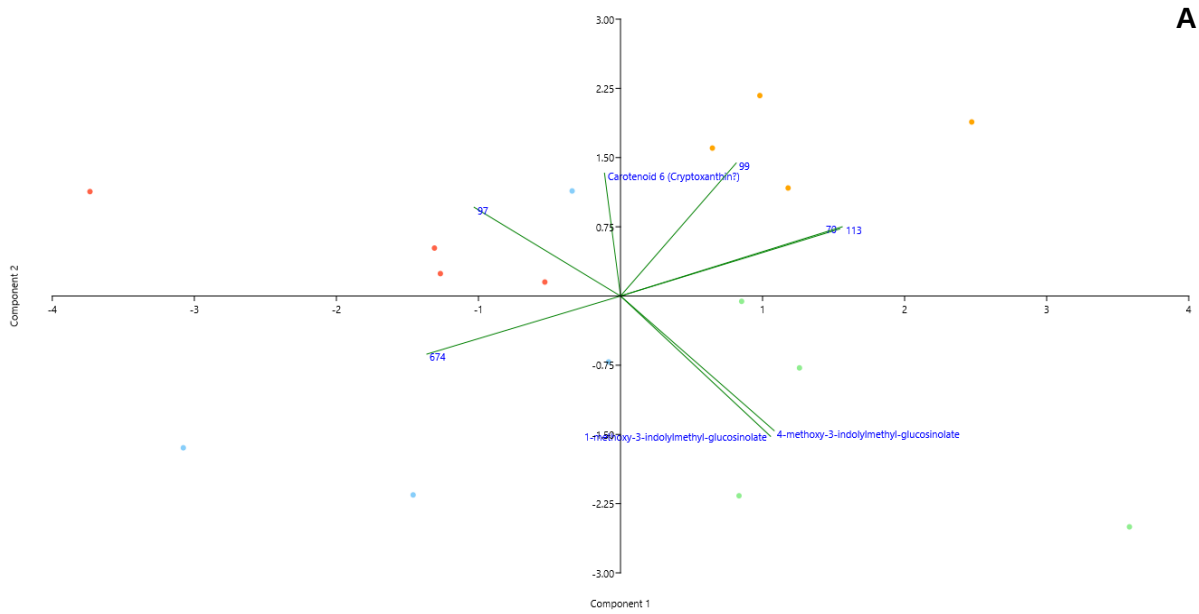


Fig. 3: Individual carotenoids, phenols and glucosinolates are sig. affected by the pre-harvest treatments (5 days). No clear conclusion can be made on which treatment is more effective in increasing the concentration of the metabolites. HPLC-PDA and LCMS data was log transformed and analyzed with One way ANOVA ($p \leq 0.05$). Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. The treatments are named according to their deviation from the control. Four replicas per treatment are used. The letters on top of the bars indicate sig. differences.



B

	PC 1	PC 2
Phenol 113	**0.4771**	0.22931
Phenol 70	**0.47183**	0.22232
4-methoxy-3-indolylmethyl-glucosinolate	0.33069	**-0.44584**
1-methoxy-3-indolylmethyl-glucosinolate	0.32289	**-0.46481**
Phenol 99	0.24902	**0.44054**
Carotenoid 6 (Cryptoxanthin?)	-0.03449	**0.40702**
Phenol 97	-0.31506	0.29397
Phenol 674	**-0.41674**	-0.19238

C

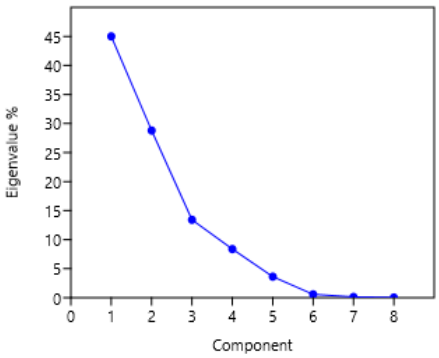


Fig. 4: A pattern based on treatments is found when the phenols, glucosinolates and carotenoids that were sig. affected (p≤0.05) by pre-harvest treatments (5 days) are analyzed with PCA in Mustard Frills Green. A: scatterplot with biplot (correlation). **B**: loadingsplot. **C**: loadings (filtered on values PC1 high to low). The different color dots in fig - A represent the treatments. Green: control treatment (R70:G10:B20 – 220 µmol m^{-2} s^{-1}), Orange: 440 µmol m^{-2} s^{-1} treatment, Red: R90:B10 treatment Blue: R60:B40 treatment. The treatments are named according to their deviation from the control. Log transformed HPLC-PDA and LCMS data was used in the PCA. Statistical testing was done with One-way ANOVA. Four replicas per treatment are used.

The third microgreen species that was analyzed in this study was Tatsoi Purple. For Tatsoi Purple only three of the many compounds that were statistically analyzed were found to be affected by the pre-harvest treatments from the pilot, all were phenols. Individual analysis of these three compounds showed no clear pattern in the effect of the treatments (Fig. 5). At this point, the effect of treatments on individual phenols is not interesting, as it is not known which compounds each phenol represents.

To gather more insight on the overall effect of the pre-harvest treatments PCA analysis was done (Fig. 6). Only PC 1 was considered during analysis, which accounted for 58% (Fig. 6 – C). Here, it is thought that PC1 represents variation caused by treatment, as batch seemed to have little effect on metabolite composition (Appendix E). The important contributors to this principal component are highlighted (Fig. 6 – B). It is shown that phenol 1153 and 757 are the largest contributors. It is shown that no clear separation between the treatments seems to be present (Fig. 6 – A). This in combination with the fact that only three of the total amount of metabolites analyzed, showed significant differences between treatments, indicates that tatsoi purple is relatively insensitive to the light treatments. Tatsoi Purple

also seems relatively unaffected by batch, indicating that the metabolite content of tatsoi is, in general, more rigorous (Appendix E). Therefore, using pre-harvest treatments to increase nutritional value in Tatsoi Purple seems less efficient.

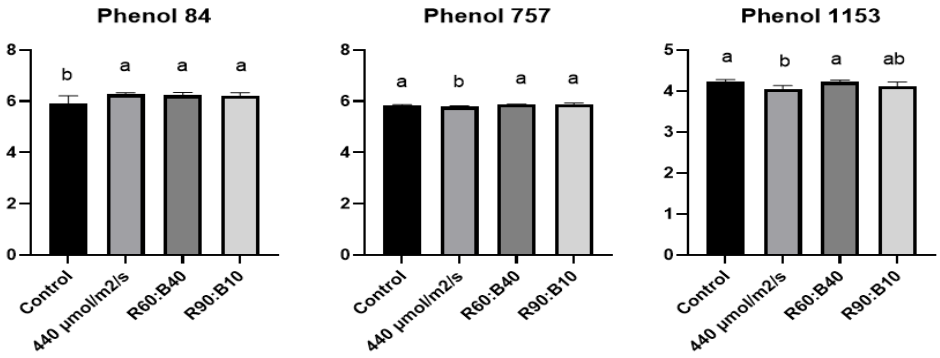
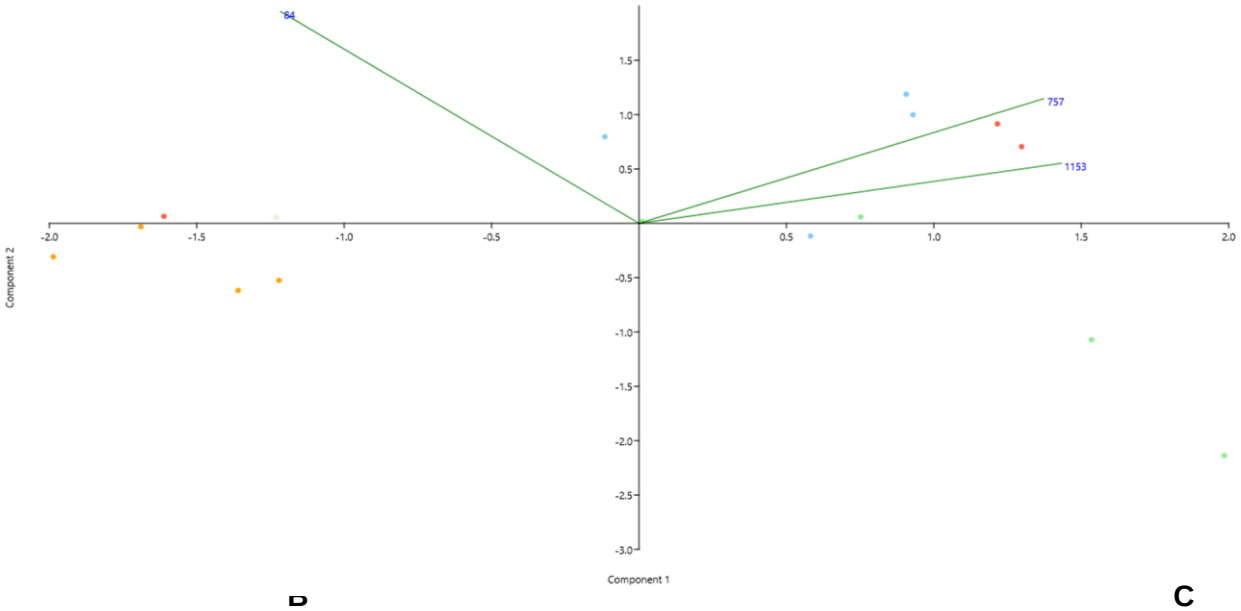


Fig. 5: Individual phenols are affected by the pre-harvest treatments (5 days). No clear pattern in effect of pre-harvest treatments on affected phenolic compounds. Data was generated with LCMS, after which it was log transformed and analyzed with One-way ANOVA ($p \leq 0.05$). Four replicas per treatment were used. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control. The letters on top of the bars indicate sig. differences.

A



B

	PC 1
Phenol 1153	**0.61562**
Phenol 757	**0.59008**
Phenol 84	-0.52232

C

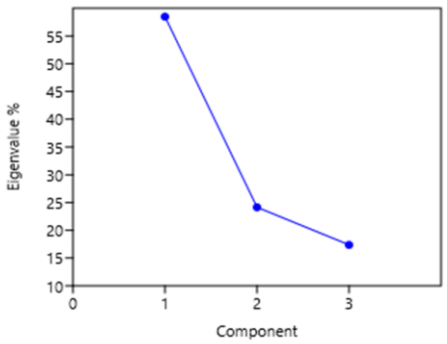


Fig. 6: No clear pattern based on treatments is found when the phenols that were sig. affected (p≤0.05) by pre-harvest treatments (5 days) are analyzed with PCA in Tatsoi Purple. A: scatterplot with biplot (correlation). **B**: loadingsplot. **C**: loadings (filtered on values PC1 high to low). The different color dots in fig - A represent the treatments. Green: control treatment (R70:G10:B20 – 220 µmol m^{-2} s^{-1}), Orange: 440 µmol m^{-2} s^{-1} treatment, Red: R90:B10 treatment Blue: R60:B40 treatment. The treatments are named according to their deviation from the control. Log transformed LCMS data was used in the PCA. Statistical testing was done with One-way ANOVA. Four replicas per treatment are used.

Lastly, PCA of the entire metabolite profile of each of the species was done and compared with the PCA of metabolites that were affected by the treatments, to gather information on the influence of the effect of treatments on the composition of the whole plant. For this, the PCA's for metabolites for which a significant effect of the pre-harvest treatments was found (A) were compared with PCA's for all compounds for which data was generated (B) (Fig. 7). It becomes clear that no distinctive pattern for treatment in the composition of microgreens (metabolite profile) can be found (Fig. 7 – A). The PCA of the metabolites where significant differences were found does show a pattern, as mentioned earlier. Therefore, the overall composition of microgreens is not severely affected by the treatments, but specific compounds, that also represent part of the composition of microgreens are. As mentioned, batch is thought to represent a large part of the variation in metabolite composition in microgreens. This is based on a comparison of the PCA of the entire metabolite profile in which samples are colored based on treatment with the same PCA in which samples are colored based on batch (Fig. 7 – A, Appendix E). Looking at the scatterplots, it becomes apparent that a clear separation of samples is present when coloration is based on batch, which is not present for treatment. Therefore, it can be concluded that batch affected metabolite concentration more severely than treatment, and that batch

did affect the overall composition of microgreens (Appendix E). The relatively large effects of the environmental inconsistencies, taken together with the effect of treatment that only becomes apparent when metabolites for which significant differences were found are analyzed, show that the use of pre-harvest treatments to optimize nutritional quality in microgreens is most valuable when the environment is stable.

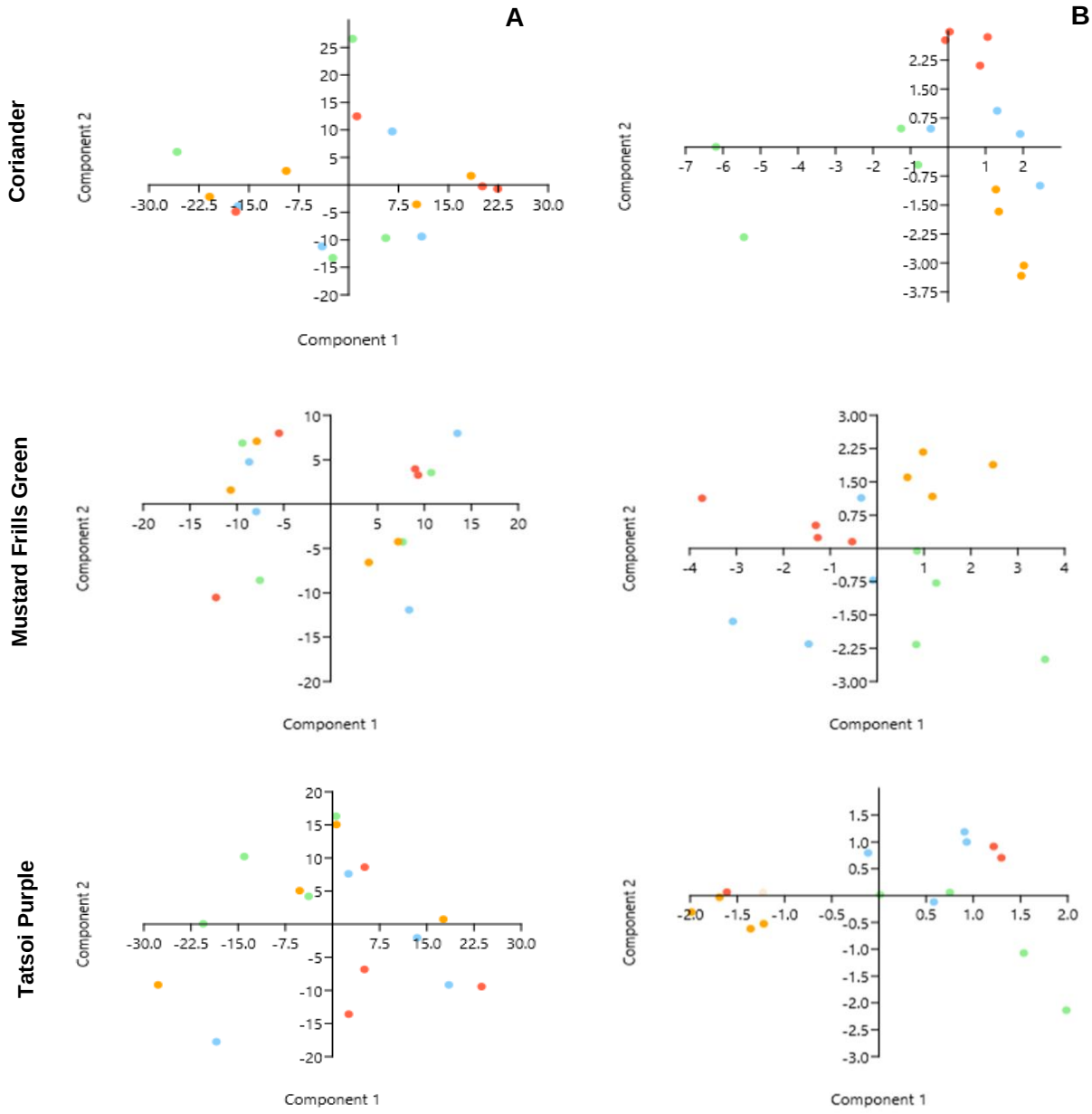


Fig. 7: Clear difference in separation of treatments in PCA with all measured metabolites (A), and only the metabolites that were affected by the pre-harvest treatments (5 days) (B) in Coriander, Mustard Frills Green and Tatsoi Purple. PCA A include: chlorophyll A and B, carotenoids, phenols, α-tocopherol, glucose, sucrose, fructose, ascorbic acid and glucosinolates. PCA B include the phenols, carotenoids and glucosinolates that were sig. affected ($p \leq 0.05$, One-way ANOVA). PCA is based on correlation. Data was generated with LCMS, HLPC-PDA and a Dionex after which log transformation took place.The different color dots represent the treatments. Green: control treatment (R70:G10:B20 – 220 µmol m^{-2} s^{-1}), Orange: 440 µmol m^{-2} s^{-1} treatment, Red: R90:B10 treatment Blue: R60:B40 treatment. Treatments are named according to their deviation from the control. Four replicas per treatment were used. In Coriander, PC1 explains 42.0% of the variance and PC2 represents 19.5% (PCA A). In PCA B, PC1 explains 37,2% of the variance and PC2 explains 24.2%. In Mustard Frills Green, PC1 explains 35.6% of the variance and PC2 explains 19% (PCA A). In PCA B, PC1 explains 45% of the variance and PC2 explains 28,8%. In Tatsoi Purple, PC1 explains 35.0% of the variance and PC2 explains 17.1%. In PCA B only PC1 is considered. PC1 here represents 58%.

3.2 Biochemical analysis main experiment

In this research project the use of pre-harvest light treatments to increase the quality of Coriander, Mustard Frills Green, and Tatsoi Purple is studied, in which nutritional value was one of the studied quality attributes. In the pilot a general overview of the effect of the 440 µmol m^{-2} s^{-1}, R60:B40, R90:B10 and control treatment on the metabolite composition of these microgreens was generated, as many compounds were analyzed with HPLC and LCMS and relatively few repetitions were used. It was concluded that carotenoids, phenols, and glucosinolates were affected by the treatments. To get a deeper understanding of specific effects of pre-harvest light treatments on metabolite concentration, in the main experiment a larger study was carried out where the same treatments were tested with more repetitions, and the effect of three new pre-harvest treatments was tested. The new treatments were the 140 µmol m^{-2} s^{-1}, the 640 µmol m^{-2} s^{-1}, and the R50:B50 treatment. Coriander microgreens were the focus of the main experiment, as it was shown that this species was affected most by the treatments in the pilot. To get a more detailed understanding of how certain plant compounds respond to pre-harvest light, the main study focused specifically on carotenoids, chlorophylls, and α-tocopherol.

First, the effect of the pre-harvest treatments on individual compounds was tested. It was found that carotenoid content was affected, while chlorophyll and α-tocopherol content was not affected, similar to the pilot (Fig. 8). In the main study, however, more carotenoids were found to be affected by the treatments. When the carotenoids that were found in the pilot and main experiment are compared, it becomes clear that the carotenoids are numbered differently. This is due to the fact that specific carotenoids were not identified in this study. Still, it is thought that the same carotenoids were found in both parts of the study. The fact that more carotenoids were found to be significantly affected in the main experiment is likely a result of the relatively small change in metabolite concentration that was caused by the treatments, resulting in the detection of more effects with a higher number of repetitions. Looking at the impact of the light treatments, it is shown that in each of the affected compounds the low light intensity treatment decreases its concentration (Fig. 8). Interestingly, the high light treatments do not seem to increase the concentration of individual carotenoids compared to the control treatment (Fig. 8). This seems to indicate that carotenoid synthesis saturates at a certain light intensity. Lastly, it seems that the control treatment and the R90:B10 treatment increase carotenoid concentration in Coriander microgreens. Regarding the effect of low light intensity on the concentration of carotenoids, the increase of carotenoid concentration for the control group could be caused by the fact that a light intensity of 240 µmol m^{-2} s^{-1} is relatively optimal for carotenoid synthesis.

To get a better overview of the more general effect of the treatments PCA was done. A PCA was done for all metabolites that were analyzed in the main experiment, being carotenoids, chlorophylls, and tocopherols, with samples colored according to treatment and batch (Fig. 9 – 1, Appendix E). In addition, a PCA was done for all carotenoids for which significant effects of the treatments were found (Fig. 9 – 2). First, the PCA of only the carotenoids for which a significant effect was found is analyzed (Fig. 9 – A, B, C 2). 69.8 % of the variation is found to be explained by PC1 (Fig. 9 – C2), which is believed to be the variation that is caused by treatment as the effect of batch seemed smaller than the effect of treatment. The reason being, that when the effect of batch was analyzed with PCA less separation was found compared to the separation of samples based on treatments (Appendix E, Fig. 9 – A1). To better understand the effects of the treatments on the concentration of the affected metabolites, the scatterplot of the PCA for these metabolites was analyzed (Fig. 9 – A2). It is found that in the scatterplot the control, R90:B10, R60:B40, and the 640 µmol m^{-2} s^{-1} group overlap for large parts, while the 140 µmol m^{-2} s^{-1} and the R50:B50 group stand out (Fig. 9 – A2). Combined with the analysis on individual compounds, it is implicated that the 140 µmol m^{-2} s^{-1} decreases carotenoid concentration. The R50:B50

might decrease carotenoid concentration as well. In addition, even though the 640 µmol m^{-2} s^{-1} group overlaps with the other treatments, it seems to be centered to the left, which could mean that high light intensity also decreases carotenoid concentration. A light intensity of 240-440 µmol m^{-2} s^{-1} seems therefore optimal for carotenoid synthesis. Looking at the PCA analysis it is unclear which treatments increase carotenoid concentration, a similar conclusion as during the analysis of individual carotenoids and the pilot experiment could be drawn, but effects are unclear (Fig. 9 – A2). The loadings plot shows that Carotenoid_25.8, Carotenoid_22.7, and Carotenoid_9.1, which is believed to be violaxanthin, are the most important contributors to PC1 (Fig. 9 – B2). It is thought that these compounds are affected most by the treatments. Lastly, the PCA for all measured compounds is analyzed to get insight into the effect of the treatments on a larger scale (Fig. 9 – A, B, C1). In this PCA, PC1 was found to explain 51,2% of the variation, while PC2 was found to explain 19.2% of the variation (Fig. 9 – C1). When the two PCA's are compared with each other it becomes clear that no large differences between the two are present regarding separation of treatments in the scatterplot and highest contributing compounds for PC1 (Fig. 9 – A1, B1). Therefore, the effect of the treatments on a larger scale is still present. It must be noted that the loadings plot of PCA 1 also shows that chlorophyll a and b contribute largely to PC1 (Fig. 9 – B1). Therefore, these compounds also affect the composition of microgreens, as it is presented here, largely.

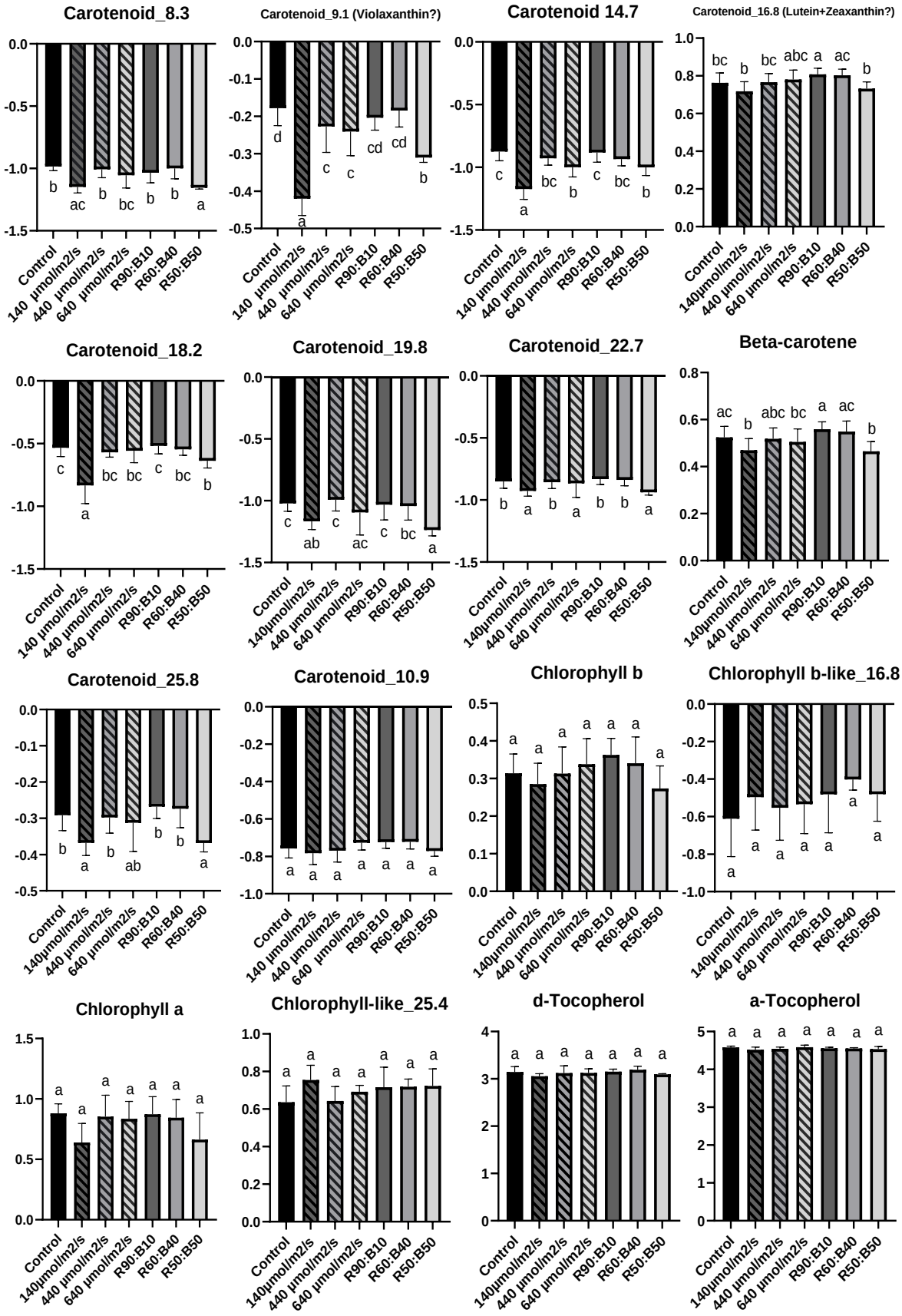
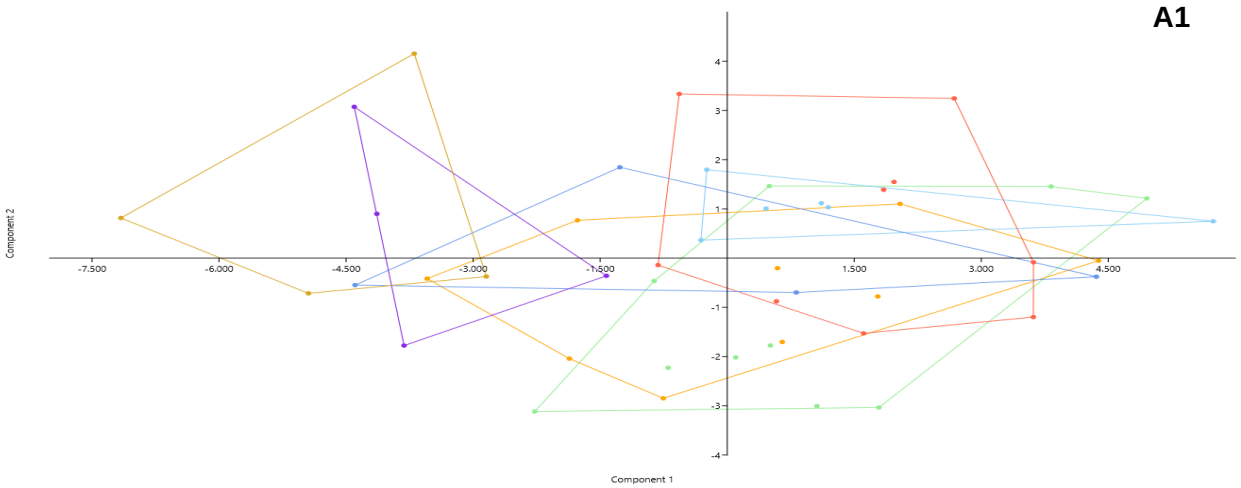
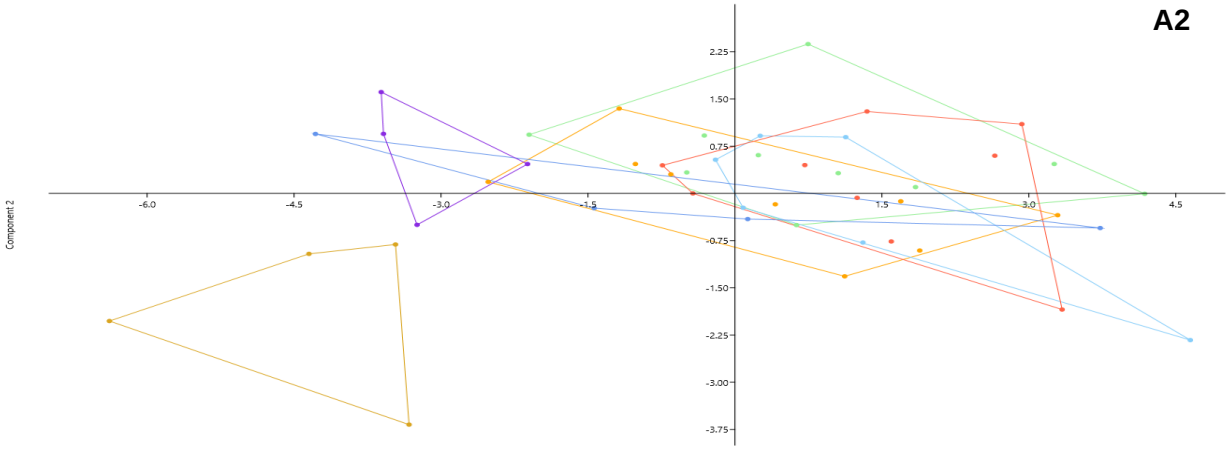


Fig 8: Pre-harvest treatments (5 days) affect the concentration of individual carotenoids, while chlorophylls and tocopherols remained unaffected. 140 µmol m^{-2} s^{-1} decreased the concentration of carotenoids, while the control and R90:B10 treatment seemed to increase it. Results of HPLC-PDA analysis of the main experiment are shown. Data was log transformed. Error bars are shown. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. A one-way ANOVA was done to determine statistical differences ($p \leq 0.05$). n=6-10 for control, R90:B10, 440 µmol m^{-2} s^{-1}, R60:B40 treatment. n=4 for 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50 treatment. Treatments are named according to their deviation from the control. The letters on top of the bars indicate sig. differences.



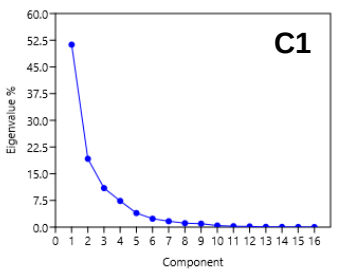


B1

Compounds	PC 1	PC 2
Carotenoid_25.8	**0.33411**	0.074001
Carotenoid_22.7	**0.32081**	0.035828
beta-Carotene	**0.31068**	0.19274
Carotenoid_9.1 (Violaxanthin?)	**0.29742**	-0.07628
Carotenoid_16.8 (Lutein+Zeaxanthin?)	**0.29339**	**0.27864**
Chlorophyll b	**0.29036**	0.042157
Carotenoid_8.3	**0.27774**	-0.11605
Chlorophyll a	**0.2715**	**-0.25819**
Carotenoid_19.8	**0.25761**	-0.13702
Carotenoid_18.2	**0.25671**	-0.088381
Carotenoid_10.9	0.24948	**0.29075**
Carotenoid 14.7	0.23495	-0.17212
a-Tocopherol	0.13527	-0.2402
d-Tocopherol	0.08253	0.22969
Chlorophyll b-like_16.8	0.014765	**0.52373**
Chlorophyll-like_25.4	-0.06481	**0.51501**

B2

Compounds	PC 1
Carotenoid_25.8	**0.38218**
Carotenoid_22.7	**0.36852**
beta-Carotene	**0.34816**
Carotenoid_9.1 (Violaxanthin?)	**0.34176**
Carotenoid_8.3	0.32959
Carotenoid_16.8 (Lutein+Zeaxanthin?)	0.32631
Carotenoid_19.8	0.3084
Carotenoid_18.2	0.30312
Carotenoid 14.7	0.2793



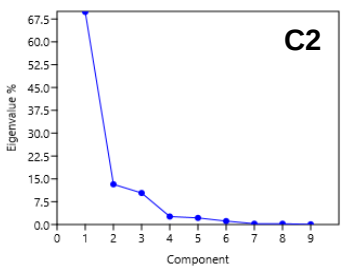


Fig. 9: A similar pattern based on treatments is found when PCA for the carotenoids, chlorophylls and tocopherols that were analyzed in the main experiment (1) was compared with PCA of the carotenoids for which a significant effect (p≤0.05) of pre-harvest treatment (5 days) was found (2). Log transformed data of Coriander microgreens was used. n=6-10 for control, R90:B10, 440 µmol m^{-2} s^{-1}, R60:B40 treatment. n=4 for 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50 treatment. Significance was tested with ANOVA. PCA is based on correlation. **A**: scatterplot with convex hulls (correlation), **B**: loadingsplot, **C**: loadings. The different color dots in fig - A represent the treatments. Green: control treatment (R70:G10:B20 – 220 µmol m^{-2} s^{-1}), orange: 440 µmol m^{-2} s^{-1} treatment, red: R90:B10 treatment, light blue: R60:B40 treatment, dark blue: 640 µmol m^{-2} s^{-1} treatment, purple: R50:B50 treatment, gold: 140 µmol m^{-2} s^{-1} treatment. Treatments are named according to their deviation from the control.

3.3 Shelf life

This project aims to increase the quality of three different microgreens species using pre-harvest treatments. Shelf life is an important contributor to quality and is valuable for both businesses and consumers. As not much is known on the effect of pre-harvest treatments on shelf life, this research project aims to gather insight on the topic. In the study, the effect of different pre-harvest treatments (Tab. 1) on shelf life was tested in Coriander, Mustard Frills Green, and Tatsoi Purple. This was done by measuring the paste at which weight loss after harvest increased. Weight loss was measured twice a week during post-harvest storage (10 °C). In this research project, the threshold after which the microgreens cannot be sold anymore is set at 1% weight loss, which was based on visual deterioration (e.g. yellowing of leaves). As environmental variation was present during the experiment (Appendix B), the effect of batch was also studied for shelf life (Appendix F).

For all species, it was found that shelf life was affected by the pre-harvest treatments (Fig. 10, Tab. 2). In Coriander, shelf life based on the 1% weight loss threshold, was approximately reached between eight and ten days (Fig. 10). The largest difference in shelf life was found between the low light treatment, where the threshold was reached after eight days, and the R90:B10 treatment, where the threshold was reached after ten days. Therefore, a 20% increase in shelf life was generated with the low light treatment (Fig. 10). Microgreens from these treatments were however not significantly different from microgreens from all other treatments. The shelf life of microgreens from the low light treatment was found to be significantly different from the shelf life of microgreens from the 440 µmol m^{-2} s^{-1}, 640 µmol m^{-2} $s^{-1,}$ and R90:B10 group (Tab. 2). On the other hand, the shelf life of microgreens from the red light treatment was found to be significantly different from the shelf life of microgreens from the R60:B40, control, and low light treatment.

In Mustard Frills Green the treatments were also found to influence shelf life. Based on figure ten, where the weight loss is presented as it was measured, microgreens from the control treatment have the lowest shelf life on average (8 days), while microgreens from the 440 µmol m^{-2} s^{-1} have the highest shelf life (12 days), a difference of 33,3%. It must be noted that statistical analysis pointed out that microgreens from the 140 µmol m^{-2} s^{-1} treatment had the lowest shelf life (Tab. 2). This difference is caused by the fact that data was ranked before it was analyzed with ANCOVA, as this was the appropriate statistical method. However, statistical analysis also pointed out that there is no significant difference in shelf life between microgreens from the control, 140 µmol m^{-2} s^{-1} and R50:B50 treatment (Tab. 2). Therefore, the shelf life of microgreens from these treatments is equal. The high light intensity treatments that increased the shelf life the most, were found to significantly improve shelf life compared to the control, R50:B50, and low light treatment (Tab. 2). It is thought that for mustard microgreens, especially light intensity affects shelf life, as the high light intensity treatments were found to increase shelf life the most, and the low light intensity treatment was found to be one of the treatments that negatively affected shelf life most (Fig. 10, Tab. 2).

In Tatsoi Purple, the blue light treatments and the 140 µmol m^{-2} s^{-1} treatment had a negative effect on shelf life (shelf life of 8-9 days), while the 440 µmol m^{-2} s^{-1} and R90:B10 treatment had a positive effect on the quality attribute (shelf life of 11 days) (Fig. 10, Tab. 2). Hence, both light intensity as spectrum seem to affect shelf life. Surprisingly, in both Mustard Frills Green and Tatsoi Purple a high light intensity resulted in a longer shelf life, while for coriander microgreens the opposite effect was found. In addition, the treatment with more red light resulted in a longer shelf life in tatsoi, and a shorter shelf life in coriander (Fig. 10, tab. 2). On that account, it seems that pre-harvest light can play a role in steering shelf life of microgreens, but treatments must be applied in a specie specific manner. Lastly, the effect of batch was analyzed. Significant differences in shelf life were found here, however,

almost all effects can be traced back to variation in the sets of treatments that were tested over time (Appendix F). The environmental variation in the cell, therefore, had a limited effect on shelf life.

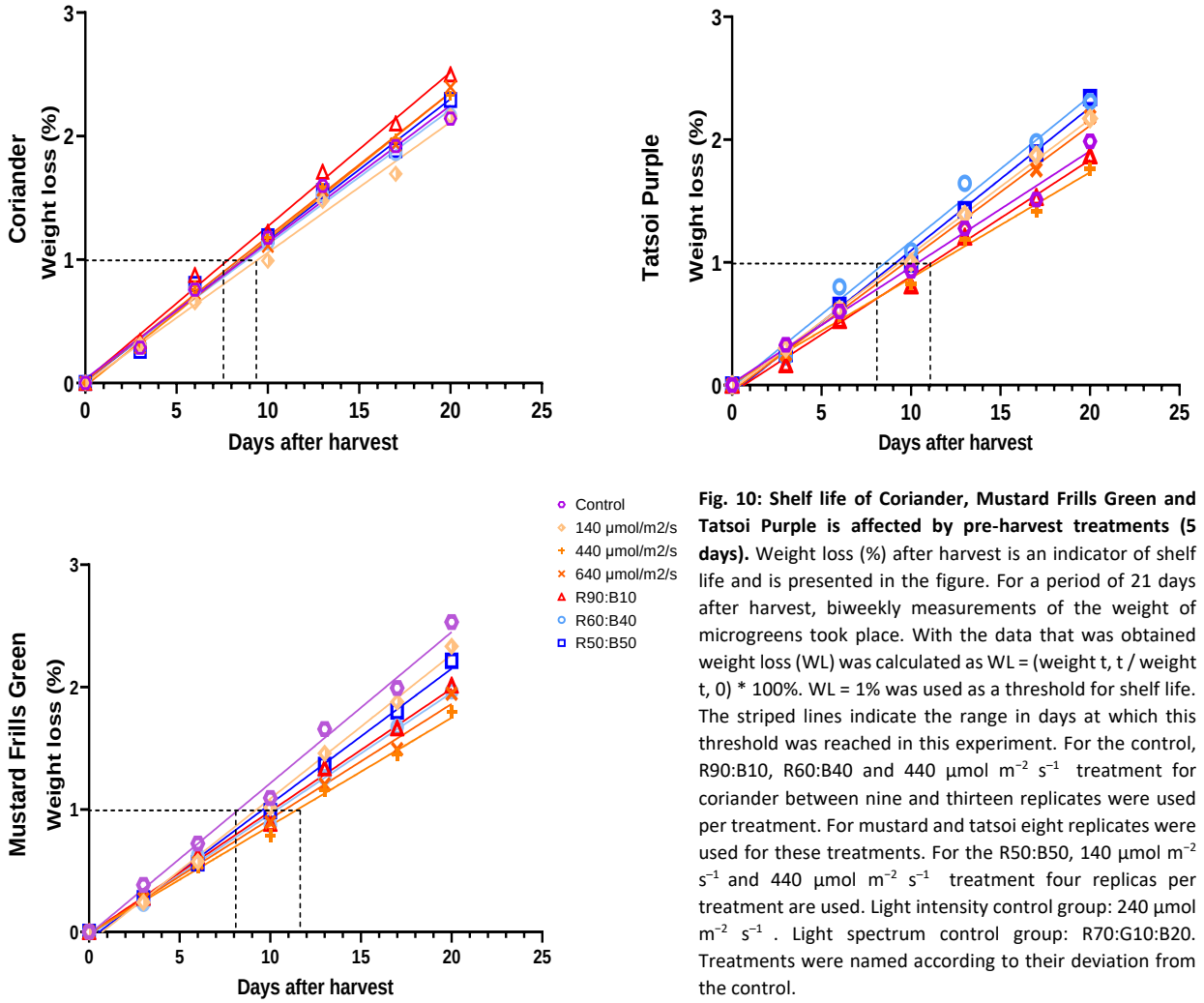


Fig. 10: Shelf life of Coriander, Mustard Frills Green and Tatsoi Purple is affected by pre-harvest treatments (5 days). Weight loss (%) after harvest is an indicator of shelf life and is presented in the figure. For a period of 21 days after harvest, biweekly measurements of the weight of microgreens took place. With the data that was obtained weight loss (WL) was calculated as WL = (weight t, t / weight t, 0) * 100%. WL = 1% was used as a threshold for shelf life. The striped lines indicate the range in days at which this threshold was reached in this experiment. For the control, R90:B10, R60:B40 and 440 µmol m^{-2} s^{-1} treatment for coriander between nine and thirteen replicates were used per treatment. For mustard and tatsoi eight replicates were used for these treatments. For the R50:B50, 140 µmol m^{-2} s^{-1} and 440 µmol m^{-2} s^{-1} treatment four replicas per treatment are used. Light intensity control group: 240 µmol m^{-2} s^{-1} . Light spectrum control group: R70:G10:B20. Treatments were named according to their deviation from the control.

Tab. 2: Overview of significant differences in shelf life in Coriander, Mustard Frills Green and Tatsoi Purple. Significance is based on analysis of the slope (treatment*days) of ranked weight loss (%) per day data with an ANCOVA model (p≤0.05). For the control, R90:B10, R60:B40 and 440 µmol m^{-2} s^{-1} treatment for coriander between nine and thirteen replicates were used per treatment. For mustard and tatsoi eight replicates were used for these treatments. For the R50:B50, 140 µmol m^{-2} s^{-1} and 440 µmol m^{-2} s^{-1} treatment four replicas per treatment are used. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments were named according to their deviation from the control. The letters behind the name of the treatments indicate significant differences.

Coriander		Mustard Frills Green		Tatsoi Purple	
Treatment	**Significance slope**	**Treatment**	**Significance slope**	**Treatment**	**Significance slope**
140 µmol/m2/s	a	440 µmol/m2/s	a	440 µmol/m2/s	a
Control	ab	640 µmol/m2/s	a	R90:B10	a
R60:B40	ab	R60:B40	ab	Control	ab
R50:B50	abc	R90:B10	ab	640 µmol/m2/s	bc
640 µmol/m2/s	bc	Control	bc	140 µmol/m2/s	c
440 µmol/m2/s	bc	R50:B50	bc	R50:B50	c
R90:B10	c	140 µmol/m2/s	c	R60:B40	c

3.4 Taste and appearance

This study investigates the use of pre-harvest light treatments to increase quality in Coriander, Mustard Frills Green, and Tatsoi Purple for the vertical farming company GROWx. As GROWx now sells most of its produce to high-end restaurants, taste and appearance are really important attributes of quality for the company. Therefore, the effect of pre-harvest treatments on the taste and appearance of the three microgreen species was tested with a taste panel consisting of chefs and employees of GROWx. Participants were asked to score several attributes on a line scale during tasting sessions for microgreens from the different treatments (440 µmol m^{-2} s^{-1}, R60:B40, R90:B10, control). Significant effects of pre-harvest treatments on the taste and appearance of the microgreens were found with One-way ANOVA based on ranks ($p \leq 0.05$).

In Coriander, microgreens that were given the R60:B40 treatment were significantly less bitter compared to microgreens of the other treatments (Fig. 11). In Tatsoi Purple, a similar effect was found. Here, it was found that microgreens that received the R60:B40 treatment were significantly sweeter than microgreens of the 440 µmol m^{-2} s^{-1} and R90:B10 treatment (Fig. 11). In Mustard Frills Green the firmness and color of the plant were influenced. Firmness was found to be significantly higher in microgreens of the control group compared to microgreens from the other treatments that were tested. Furthermore, it was found that microgreens from the high light intensity treatment had a higher firmness compared to microgreens of the R90:B10 treatment (Fig. 11).

In addition to effects on the taste of microgreens, an effect on appearance was found in Mustard Frills Green. It became clear that microgreens of the control treatment were significantly more purple compared to the other treatments (Fig. 11). Added to that, it was found that microgreens of the R60:B40 treatment were significantly less purple. A similar pattern was found for the tint of green. Microgreens from the control group had a significantly darker color, and microgreens from the R60:B40 treatment had a significantly lighter color when they were compared with the other microgreens. It must be noted that the number of participants in this study was relatively low (4-6). As many participants reported large differences between microgreens from the different groups, more significant differences in taste would likely be found when more people would have participated in the study. Next to the attributes shown in figure eleven, in this experiment overall taste experience and overall liking were scored. No significant differences in preferences were found. Still, the effects that pre-harvest light treatments have on taste and appearance are valuable as they indicate potential to target specific taste attributes that are important for customers.

Fig 11: Taste is sig. affected by pre-harvest treatments (5 days) in Coriander, Mustard Frills Green and Tatsoi Purple. In Coriander R60:B40 microgreens were less bitter. In Tatsoi Purple R60:B40 microgreens were sweeter. In Mustard Frills Green the control group had a higher firmness, followed by the 440 µmol m^{-2} s^{-1} treatment. In addition, control microgreens were darker in color and had more purple in them, and R60:B40 microgreens were lighter green and had less purple in them. Statistical testing was done with ANOVA ($p \leq 0.05$). Light intensity control group: 240 µmol m^{-2} s^{-1} . Light spectrum control group: R70:G10:B20. Treatments were named according to their deviation from the control. Number of participants for each microgreen session: coriander 6, mustard 6, tatsoi 4 = n. Treatments are named according to their deviation from the control. ∗ means that a sig. effect of the treatments was found for the attribute.

3.5 Yield attributes

The main goal of this experiment was to increase microgreen quality with pre-harvest light intensity and light spectrum treatments (Tab. 1). For the implementation of these treatments, it is important that yield is not negatively affected, as next to quality yield plays a major role in creating revenue for the farm. Therefore, the effect of the studied treatments on yield attributes was analyzed. To get a complete overview of the effect of the treatments fresh weight (g), dry weight (%), and hypocotyl length (cm) of the microgreens were studied. No significant effect of the treatments on these attributes was found in Coriander, Mustard Frills Green, and Tatsoi Purple, which is beneficial for the use of pre-harvest treatments to increase quality (Fig. 12). When the same attributes were analyzed for an effect of batch some significant differences were found (Appendix G). Therefore, batch, as a result of inconsistencies in climate (Appendix B) and water gift, had a larger effect on fresh weight, dry weight, and hypocotyl length than the pre-harvest treatments that were tested. Effects of the treatments on yield attributes could therefore be found in a more stable environment.

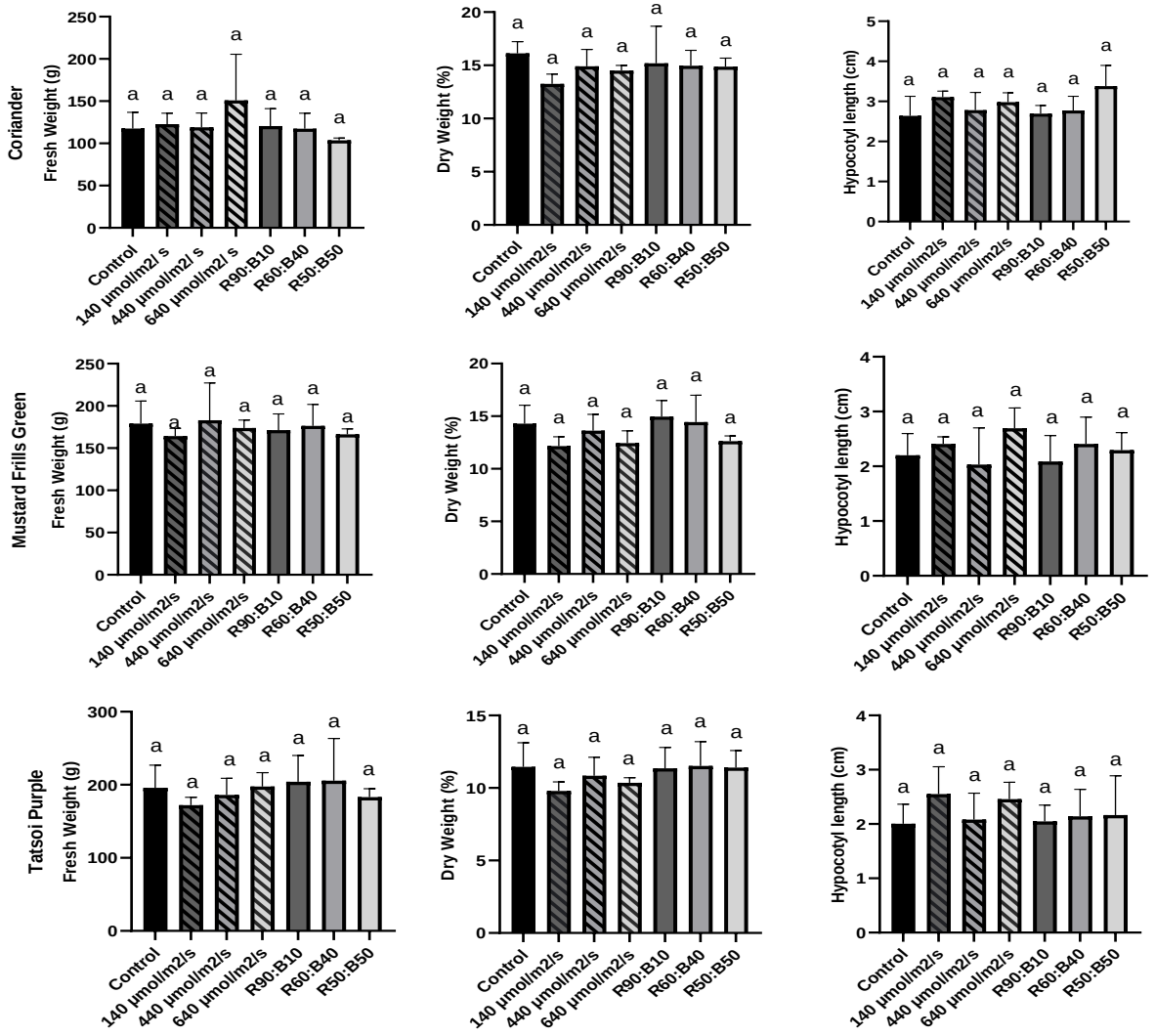


Fig. 12: Fresh weight (g), dry weight (%) and hypocotyl length (cm) of Coriander, Mustard Frills Green and Tatsoi Purple microgreens are not affected by the pre-harvest treatments (5 days). Fresh weight was measured by weighing all microgreens from a gutter directly after harvest. Dry weight was measured by weighing the microgreens before and after they were dried in an oven for 24-48h at 105 °C. With this data the dry weight % was calculated ((dry weight/ fresh weight) *100%). For hypocotyl length the length from the beginning of the stem till the hypocotyl was measured with a ruler directly after harvest. The length of five microgreens per gutter was averaged and used as one experimental unit. Statistical analysis was done with One-way ANOVA and Brown-Forsythe ($p \leq 0.05$). Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. For the control, R90:B10, R60:B40 and 440 µmol m^{-2} s^{-1} treatment for coriander between nine and thirteen replicates were used per treatment. For mustard and tatsoi eight replicates were used for these treatments. For the R50:B50, 140 µmol m^{-2} s^{-1} and 440 µmol m^{-2} s^{-1} treatment four replicas per treatment are used. The letters on top of the bars indicate sig. differences.

3.6 Model

A lot of time and costs are associated with an extensive research project like this one. As many similar projects are still needed to make full use of the potential that vertical farming offers regarding the optimization of the growth environment per species, it is interesting to put effort towards the development of a tool to speed up the research process. Development of PLS regression models based on VIS-NIR spectrometry that can predict the concentration of certain plant compounds could potentially be used to this end. When such models would be implemented in the automated growing system of GROWx, tests on the optimal environment for any crop or crop qualities could then be performed in an easy, cost-effective manner. It must be noted, however, that for the use of such models to increase quality attributes, also more knowledge on the interaction between plant

compounds and human health, shelf life, taste, and appearance must be present. From the reasons presented above, this study combined research on the use of pre-harvest light to increase quality in microgreens with the development of PLS models. First, models were developed with the metabolite data that was obtained from the pilot study, to get a general idea about the use of PLS modeling for the prediction of concentrations of interesting plant compounds. Analysis of the metabolites during the pilot was done with LCMS, HPLC-PDA, and a Dionex. During this phase, PLS regression models were developed for sugars, ascorbic acid, glucosinolates, phenols, carotenoids, chlorophylls, and α-tocopherol, and dry weight and were made for Coriander, Mustard Frills Green and Tatsoi Purple. Next, models were developed with metabolite data from the main experiment, that was focused on the effect of pre-harvest treatments on carotenoids, chlorophylls, and tocopherols in Coriander. Here, analysis was done with HPLC-PDA. For the development of the model, many repetitions were generated. As a result, for these compounds, an accurate assessment could be made regarding the potential of the model and its further development with the methods used during this study. In the following section, first, the results of the models that were developed during the pilot are discussed.

The accuracy of the PLS models that were developed during the pilot experiment varied strongly per compound, and species (Fig. 13). A general trend that was observed however was that the R^2, which indicates the accuracy of the model, was a lot higher when the model was based on the three species together than when the model was based on one of the species (Fig. 13). This makes that the PLS regression acts mostly to discriminate between the species, rather than correctly predict the variation in sugar content within one species. For some compounds, a model that can give a general concentration of a compound for a range of species can be quite interesting (e.g. ascorbic acid), for others it is not (e.g. unknown carotenoid). It was anticipated that the R^2, and thus the accuracy of the model, would increase when more repetitions were used for the development of the model. In the main experiment, more repetitions were used, thus a better assessment of the potential of the models that were developed could be made. Unfortunately, the increase in accuracy that was anticipated was not found (Fig. 14). The development of PLS regression models to predict the concentrations of interesting compounds with the methods used in this study was therefore unsuccessful. It is thought that this is due to the relatively low accuracy of the HPLC-PDA data, caused by experimental error, which now seems insufficient for the development of a model (S. Hageraats, *personal communications*, 14-7-2021). For the repeated measurements of the internal standard namely quite some variation was observed. In Appendix H, the development of the model is reported in a more detailed manner.

In the main experiment also Linear Discriminant Analysis (LDA) was done. The model showed the ability to accurately classify microgreens for the pre-harvest treatments they received based on VIS-NIR data (Fig. 15). This supports the analysis of biochemical data that was discussed earlier in this research report, in which it was concluded that pre-harvest treatments did affect the concentration of metabolites. The high classification accuracy based on treatments shows that VIS-NIR data can detect very small differences between groups and supports the idea that stronger effects of the treatments are present than could be shown in this study.

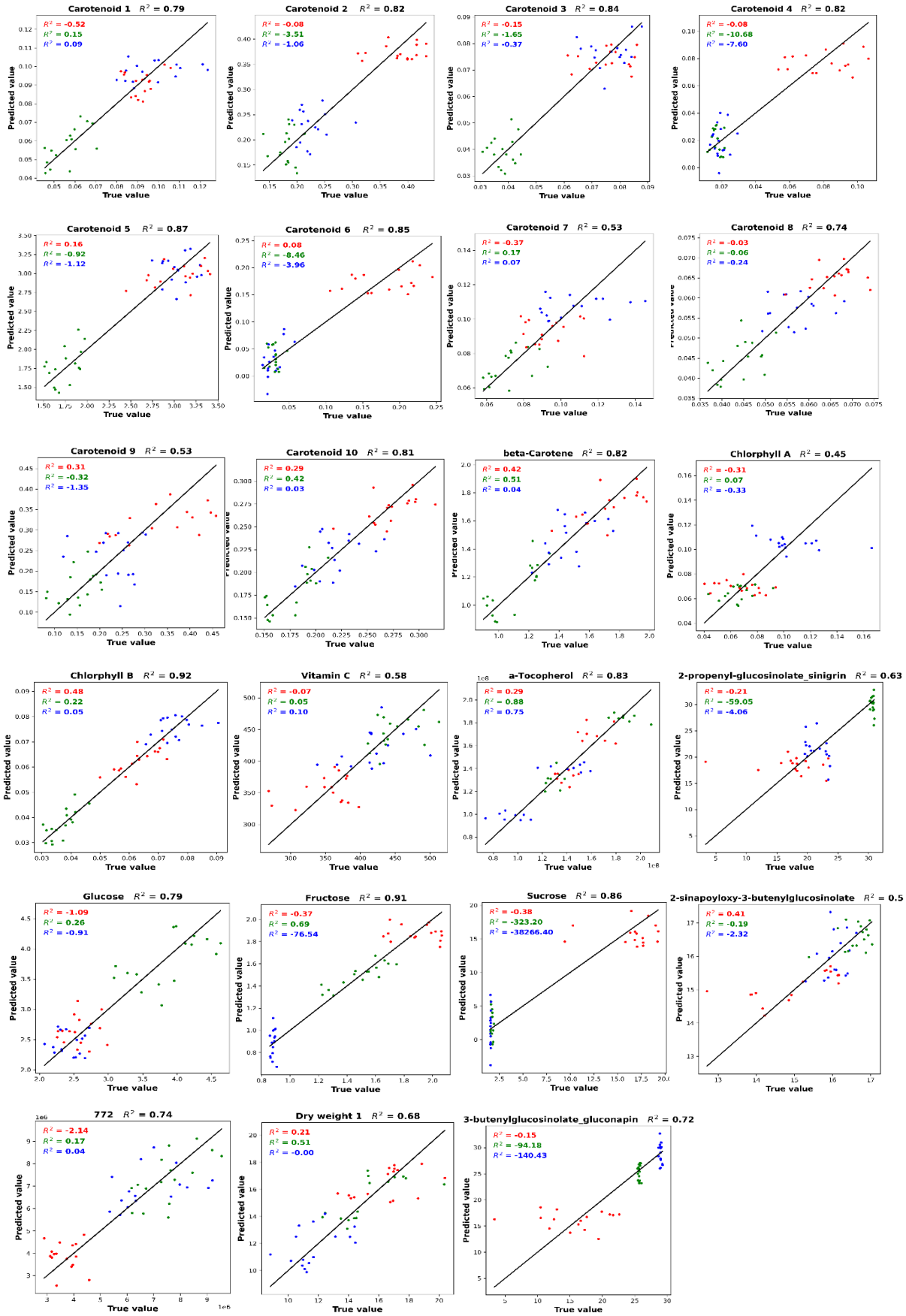


Fig 13. PLS regression models that predict the concentration of various compounds based on VIS-NIR spectrometry show potential (low n, moderate R^2 values). Coriander (red), Mustard Frills Green (green) and Tatsoi Purple (blue). Moderate R^2 values are found when all species are taken together. R^2 for models based on one species are low. Accuracy of model varied strongly per compound and species and was expected to increase with more replicates. Compound concentrations, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), were analyzed with LCMS, HPLC-PDA and a dionex. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

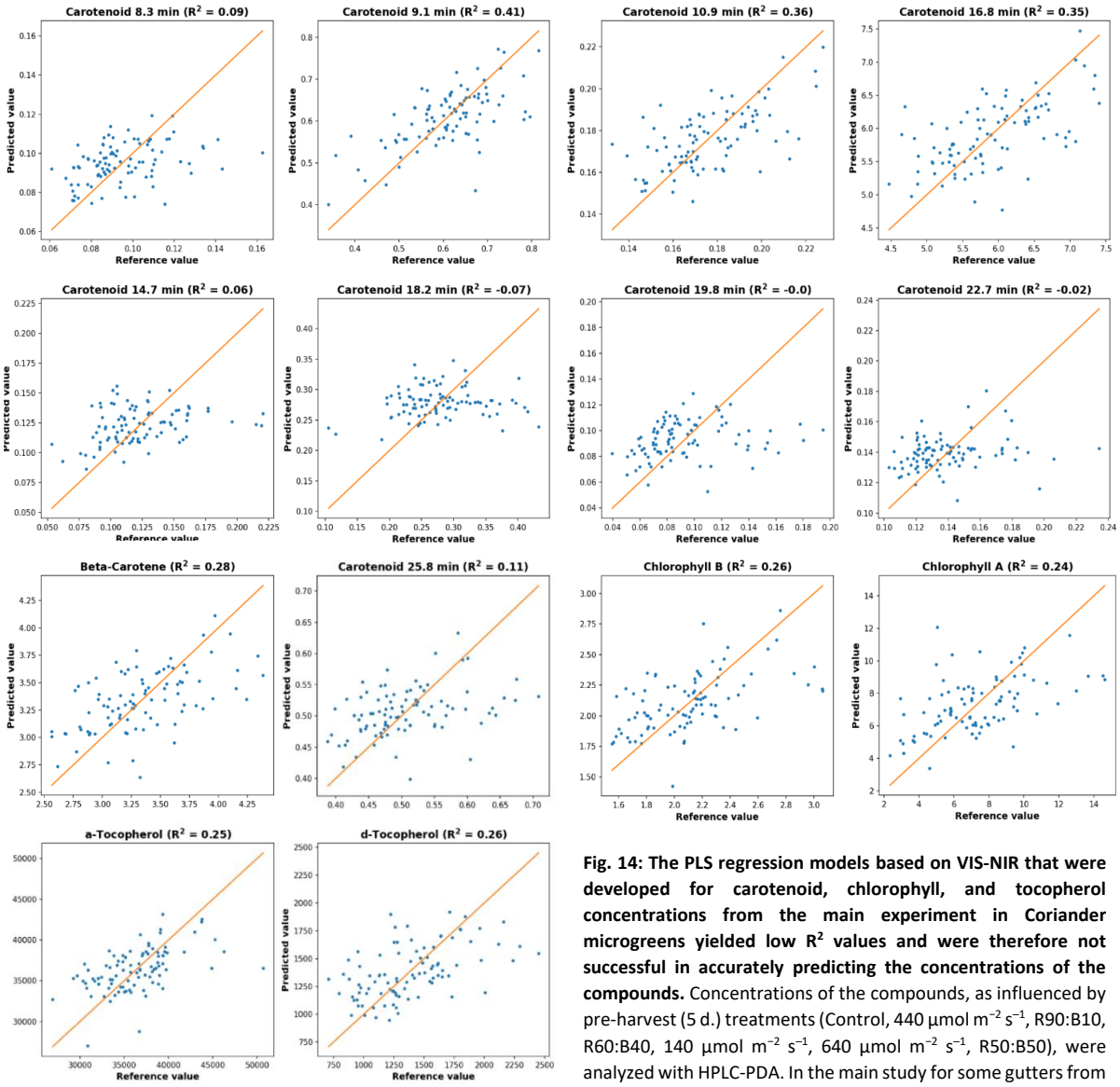


Fig. 14: The PLS regression models based on VIS-NIR that were developed for carotenoid, chlorophyll, and tocopherol concentrations from the main experiment in Coriander microgreens yielded low R^2 values and were therefore not successful in accurately predicting the concentrations of the compounds. Concentrations of the compounds, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40, 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50), were analyzed with HPLC-PDA. In the main study for some gutters from the first four treatments three samples per gutter were analyzed, as more data was needed for the development of the model. This resulted in n=19-22 for these treatments. For the last three treatments n=4. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group.

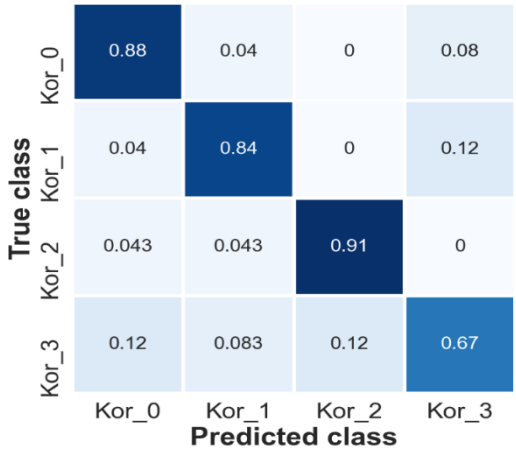


Fig. 15: Linear Discriminant Analysis (LDA) shows accurate separation of pre-harvest treatments (5 days). The control treatment (0), 440 µmol m^{-2} s^{-1} treatment (1), R60:B40 treatment (2) and R90:B10 treatment (3) were tested. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Per treatment 19-20 repetitions were used.

3. Discussion

Vertical farming systems could supply urban areas with fresh produce (Graamans et al., 2018). Therefore, vertical farming can contribute to a sustainable food system. Optimization of the vertical farming system is however important, as this can lead to improved product quality (Kozai et al., 2015). GROWx, a vertical farming company that cultures microgreens, has therefore set up a project called GROWx 2.0, which aims to optimize the vertical farming system with the use of AI and robotics. This research project is part of this project and specifically focuses on the use of pre-harvest light treatments to increase microgreen quality. For GROWx, product quality is really important as the company now sells its produce to high-end restaurants. The quality of the microgreens that the company produces creates attractiveness for these buyers. Pre-harvest treatments could potentially increase microgreen quality further, which could increase the position of GROWx within the market (Gómez & Jiménez, 2020; Samuoliene et al., 2012). Many studies have been done about the effect of light treatments on the quality of crops, but studies that focus on pre-harvest treatments, which have increased potential due to their easy implementation and low costs, are limited. In addition, the studies that were done usually focused on one aspect of quality. For GROWx, the effect of pre-harvest treatments on all important quality aspects is interesting. Even more so, because quality attributes of a crop seem to be related to each other, specifically to nutritional compounds in the plant. To get insight into the potential that pre-harvest light treatments offer to increase quality, an experiment was designed where the effect of six different pre-harvest treatments (5 days) was tested on nutritional value, shelf life, taste, appearance, and yield in Coriander, Mustard Frills Green and Tatsoi Purple microgreens. The treatments that were tested were the 440 µmol m^{-2} s^{-1}, R60:B40, R90:B10, control treatment, the main focus of this study, and the additional 640 µmol m^{-2} s^{-1}, 140 µmol m^{-2} s^{-1} and R50:B50 treatment. The control treatment had a spectrum of R70:G10:B10 and a light intensity of 240 µmol m^{-2} s^{-1}. The light intensity treatments had the same spectrum as the control treatment and vice versa. It was hypothesized that all quality attributes would be affected. Mostly blue light and high light intensity were expected to increase microgreen quality, but it was also thought that they could be related to yield loss (Alrifai et al., 2019; Gómez & Jiménez, 2020). In addition, it was expected that red light or low light intensity could also increase some quality attributes, as this was shown in earlier studies (Meas et al., 2020; Samuoliene et al., 2012). Lastly, species-specific effects were thought to be present (Zhang et al., 2020). From the start of this research project, it was known that more research must be done to optimize the light environment in the vertical farm. As this process is costly, this research project was used to develop a model that predicts the concentration of interesting plant compounds, which could reduce the costs and time needed for research projects like this one.

The pre-harvest treatments were found to affect the concentration of several nutritional compounds, the shelf life, the taste, and the appearance of all three microgreens species, as was hypothesized. This makes sense, as light plays a large role in various processes in the plant. For starters, it plays a role in photosynthesis which is related to growth. Additionally, among many other things, light plays a role in biosynthetic pathways for metabolite production. Regarding this, it is known that different colors of light stimulate these pathways in different ways (Jung et al., 2021). Most nutritional compounds that were studied in this research project have antioxidant properties, and therefore play a role in preventing oxidation damage. Plants experience oxidation damage during stress. Stress could be caused by a high light intensity or by light with a high energy, like UV or blue light (Alrifai et al., 2019). The effects of pre-harvest light treatments, that were found in this project, are likely related to a combination of photosynthetic effects, and effects of light on various pathways in the plant. In turn,

differences in metabolite concentration that were found here are probably partly caused by stress responses of the plant (or absence of) as a result of light treatments. The differences that were found for shelf life, taste and appearance, are likely the result of the correlation between these quality attributes and compounds in the plant that were influenced by the treatments. In this experiment, it was found that each species reacts differently to the light treatments. It is thought that this is due to the variation in biosynthetic pathways in species (Jung et al., 2021). For carotenoids for instance it was found that different light treatments increased the expression of FtPSY, FtLCYB, FtCHXB, FtCHXE, FtLCYe, and FtZEP genes, that are related to carotenoid synthesis, in buckwheat sprouts, while in another study the CitPSY, CitZDS, CitPDS, and CitLCY genes varied in expression based on light treatments, which are also associated with carotenoid synthesis, in citrus species (Jung et al., 2021). Lastly, in this research, no effect of the pre-harvest treatments on fresh weight, dry weight, and hypocotyl length was found, even though it is widely known that light can influence these attributes. However, pre-harvest light treatments have a short application period, which likely reduces the impact of these treatments on yield. In the next section, the effects of the pre-harvest light treatments will be discussed in more detail.

The effects of pre-harvest light treatments on the concentration of nutritional compounds that were found during the pilot were more severe in Coriander and Mustard Frills Green compared to Tatsoi Purple (Fig. 7). In Tatsoi Purple the effect of batch was also minimal, which leads to the conclusion that in this experiment Tatsoi Purple was less sensitive to environmental factors (Appendix E). Although the concentration of more nutritional compounds was affected in Coriander and Mustard Frills Green, the effect of pre-harvest treatments on nutritional value remained relatively low in these species as well (Fig. 7). The compounds affected are mostly phenols and carotenoids. In Mustard Frills Green also two glucosinolates were affected. It was hypothesized that the other compounds that were analyzed in this study would also be affected by the light treatments, as similar studies showed that such effects exist (Appendix I). Research projects with a focus on coriander microgreens are limited, but some studies on mustard and tatsoi microgreens have been done. In these studies, the light treatments tested caused a relatively large effect on the concentration of many nutrients in microgreens. A study performed on tatsoi, for instance, found that the ascorbic acid content (Vitamin C) was increased 3.8 times when microgreens that received a light intensity of 110 µmol m^{-2} s^{-1} were compared with those that received 220 µmol m^{-2} s^{-1} (Samuoliene et al., 2013). In addition, sucrose concentration was greatly affected in this study. In tatsoi sucrose concentration increased 9.5x at 545 µmol m^{-2} s^{-1} compared to 220 µmol m^{-2} s^{-1} (Samuoliene et al., 2013). Added to that, research projects show that light is involved in the biosynthetic pathways of many of the studied compounds, indicating that a change in the light environment would influence the concentration of these compounds (Zhang et al., 2020). In this research project, no significant effect of the treatments was found for sugars, chlorophyll A and B, ascorbic acid, α-tocopherol, and other carotenoids, phenols, and glucosinolates. As there is a lot of evidence that these compounds are affected by light treatments, it is thought that differences between treatment groups were small in this experiment and therefore not significant for many compounds. This idea is supported by the fact that LDA analysis showed accurate classification based on treatments, using spectrometry data of tested microgreens (Fig. 15). An explanation for this finding could be the short exposure period of the treatments. Most studies focused on the effect of light during the whole growth period, which likely increased the magnitude of effects of the treatments on nutritional value. However, it is thought that the effect of batch on nutritional value, which represents the effect of environmental variation during the experiment, is the main reason that a limited number of

compounds was found to be affected by the treatments (Appendix E). It is thought that the effect of batch overshadows the effect of the pre-harvest treatments on nutritional value in this experiment, as the effect of batch was found to be larger than the effect of pre-harvest treatments (Appendix E, Fig. 7 – A).

Still, several effects of the pre-harvest treatments on nutritional value were found. During the pilot experiment, it was found that in Coriander microgreens individual phenols were mostly increased by either the treatment with more red or more blue light (Fig. 1). Especially red light seemed effective to increase the concentrations of the metabolites in the Coriander, which contradicts the hypothesis that metabolites are mostly increased by blue light. Although it must be noted that no significance analysis was done that indicates that red light improves metabolite concentration more than blue light. Similarly to these results, recent research shows that both red and blue light can increase the synthesis of phenols (Jung et al., 2021). Another study, concluded in line with this, that phenol synthesis is optimal when a combination of red and blue light is used. The study mentioned that this is likely caused by the fact that chlorophyll a and b both absorb red and blue wavelengths, which increases the synthesis of compounds in general and by the fact that red and blue light together can modulate different pathways for different syntheses (Alrifai et al., 2019). In Mustard Frills Green and Tatsoi Purple, no clear pattern was found when individual phenols were analyzed (Fig. 3, Fig. 5). It, therefore, seems that the synthesis of each phenol was optimal under different light conditions. The concentration of the affected glucosinolates was found to be significantly higher for microgreens from the control treatment, compared to the R90:B10 and the 440 µmol m^{-2} s^{-1} treatment. It is not clear why glucosinolate concentration is affected in this way. Further research on the effects of light treatments on glucosinolate concentration must be done to understand this, especially because of the role that this compounds play in cancer prevention and the taste of crops (Alrifai et al., 2019; Steinbrecher et al., 2009). In the pilot study, in addition to phenols and glucosinolates, several carotenoids were found to be affected by the pre-harvest treatments. During the pilot, no clear pattern for these effects was found (Fig 1, Fig. 3). But, in the main experiment more repetitions were used, and insight into the general effects of light treatments on carotenoid concentration was generated.

When in the main experiment chlorophylls, tocopherols and carotenoids were investigated, only carotenoids were found to be affected by the treatments. It was found that the low light intensity treatment decreased carotenoid content (Fig 8, Fig. 9). One might expect that high light intensity than increases carotenoid concentration, but such effects were not found. No other clear trends in the effect of the pre-harvest treatments were found. In the review of Alrifai et al. (2019), it was concluded light intensity can directly impact the concentration of carotenoids and other pigments via stimulation of enzyme activity through light-induced stress. This effect likely relates to the photoprotective role of carotenoids (Zhang et al., 2020). Alrifai et al. (2019) also concluded that light intensity has a larger effect on carotenoid concentration than light spectrum, which was also the case in this study. Literature shows that the optimum light intensity for carotenoid synthesis is highly dependent on the species. Research by Brazaityte et al., (2015) showed that the concentration of carotenoids was highest when a light intensity of 330-440 µmol m^{-2} s^{-1} was used in red pak choi and tatsoi, while for mustard the highest concentration of carotenoids was found with a light intensity of 110-220 µmol m^{-2} s^{-1}. The highest irradiance level that was studied in this project was 545 µmol m^{-2} s^{-1}. Under these light conditions, the concentration of carotenoids decreased in all species (Brazaityte et al., 2015). In this study, a light intensity between 220-440 µmol m^{-2} s^{-1} seems to be optimal to increase carotenoid concentration. No effect clear effect of light spectrum was found in this study. Several other studies

did find an effect of light spectrum on carotenoid concentration (Alrifai et al., 2019; Brazaityte et al., 2015; Zhang et al., 2020). In these studies, it was for instance found that blue light, yellow light, or white light treatments could increase the concentration of carotenoids depending on the species. However, similar to this study (Fig. 9), light spectrum did not always clearly affect carotenoid content (Meas et al., 2020). This study showed that nutritional value can be influenced by pre-harvest treatments. However, it is also shown that other environmental factors influence the attribute to a large extend. Therefore, pre-harvest treatments to increase nutritional value are considered to be most useful in a stable environment.

Next to improving nutritional quality, this project tried to investigate the use of pre-harvest treatments to improve shelf life. Few research projects have investigated the effect of pre-harvest light on shelf life. However, recently two studies on the topic have been done. It was shown that the shelf life of lettuce was increased with high light intensity treatments and that the shelf life of rocket and spinach increased when a spectrum with 35% blue light was used (Min et al., 2021; Nicole et al., 2019). In this research project, pre-harvest treatments were also found to affect shelf life when weight loss (%) after harvest was analyzed (Fig. 10, Tab. 2). Species-specific effects were found, which might hamper application of these treatments to improve shelf life. In Coriander the 140 µmol m^{-2} s^{-1} treatment increased shelf life the most and was found to be significantly different compared to the 440 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1} and R90:B10 group (Tab. 2). The R90:B10 treatment was found to have a negative effect on shelf life compared to the control treatment, the low light intensity treatment, and the R60:B40 treatment. To get a better idea of the use of these treatments to extend shelf life, a 1% weight loss threshold was set, after which microgreens were considered uneatable due to visual deterioration. Figure ten was used to determine after how many days microgreens from the treatments researched this threshold on average. The results differed slightly from the statistical analysis which was based on ranked weight loss data. A 20% (two-day difference) increase in shelf life was found in Coriander when the low light treatment was compared with the R90:B10 treatment (Fig. 10). As the shelf life of microgreens is usually very short, an increase in shelf life of 2-3 days, as found in this study, can already be of great importance for the consumer or vertical farm (E. Kolmer, *personal communications*, 11-5-2021). On the contrary, in Mustard Frills Green and Tatsoi Purple high light intensity increased shelf life. In Mustard Frills Green the 440 and 640 µmol m^{-2} s^{-1} treatment increased shelf life compared to the 140 µmol m^{-2} s^{-1}, R50:B50 and control treatment. The low light intensity treatment resulted in the shortest shelf life. In figure ten the control treatment seems to have the shortest shelf life. Based on the figure, a 33% (4-day difference) increase in shelf life is found for the high light intensity treatments compared to the control treatment. No statistical difference between the control treatment and 140 µmol m^{-2} s^{-1} treatment is found. Therefore, both have a similar effect on shelf life. In Tatsoi Purple the 440 µmol m^{-2} s^{-1} and R90:B10 treatment increased shelf life compared to all treatments but the control treatment. The blue light treatments and the low light treatment decreased shelf life in this species. A difference in shelf life of approximately three days (27%) was present between the highest performing treatments and the lowest-performing ones. These results implicate that light intensity has a larger effect on shelf life than light spectrum. In Tatsoi Purple and Mustard Frills Green high light intensity seems to increase shelf life. The same effect was found by Min et al. (2021). In the study, it was concluded that the increase in shelf life that was found under high light pre-harvest conditions was due to an increase in ascorbic acid and carbohydrate content before harvest, which postponed deteriorative processes that are connected to senescence. In addition, initial ascorbic acid affects shelf life via its anti-browning effect (Min et al., 2021). In this study, no significant difference in ascorbic acid

and dry weight (which relates to carbohydrate content) was found. Therefore, in this study, such a conclusion cannot be drawn. This could be due to the fact that the effect of batch overshadowed the effect of treatment for these compounds. However, more research must be done to determine this. As mentioned, another study that researched the effects of pre-harvest treatments on shelf life showed that using a spectrum with 35% blue light increased shelf life in rocket and baby spinach (Nicole et al., 2019). In this research project, the blue light treatments had the largest impact on shelf life in Tatsoi Purple, where they decreased shelf life. Accordingly, also the effect of blue light on shelf life seems to be species-specific. In Coriander, the red light treatment increased shelf life and no significant differences were found between the blue light treatments and the low light treatment, which generated the highest shelf life. Therefore, a similar effect of blue light as described by Nicole et al. (2019) could be present in Coriander. No explanation for the effect of blue light on shelf life was described, but based on the study of Min et al. (2021) and the species-specific effects of light treatments on nutritional quality, initial nutritional and dry matter content could play a role. Lastly, it must be noted that some results in the shelf life study are inconsistent. In Tatsoi Purple for instance the 440 µmol m^{-2} s^{-1} treatment increases shelf life the most, while the 640 µmol m^{-2} s^{-1} treatment is less effective. In this study, the shelf life of the 640 µmol m^{-2} s^{-1} group differs significantly from the 440 µmol m^{-2} s^{-1} group but does not significantly differ from microgreens from the 140 µmol m^{-2} s^{-1} treatment. Such effects seem unlikely when light intensity influences shelf life, however a non-linear relationship between light intensity and shelf life could be present.

The last quality attributes that were investigated in this research project were taste and appearance. These attributes were studied in a separate study for which a taste panel consisting of employees of GROWx and chefs was set up. In this study, it was found that blue light decreased the bitterness in Coriander and increased the sweetness in Tatsoi Purple (Fig. 11). Few studies on the effect of light on the taste of microgreens have been done. One study on the topic that has been done focused on the effect of light on the concentration of compounds that are related to taste such as phenols, essential oils, and secondary metabolites (Litvin et al., 2020). Phenols specifically are related to bitterness, sourness, and astringency (Caracciolo et al., 2020). The study found that blue light increased the concentration of these taste-related compounds in basil, dill, and parsley (Litvin et al., 2020). It was therefore expected that blue light increases bitterness instead of decreasing it, which was found in this study. However, in the study of Litvin et al. (2020) only blue light percentages similar to the R90:B10 and the control treatment were used, while in this study a spectrum with a higher percentage of blue light was used. In addition, in this research project, it is concluded that phenol concentration can also be increased with red light, depending on the specific phenol and the species, which was supported by the analysis of Jung et al. (2021). For Coriander specifically, it was indicated that red light increased the concentration of phenols more than the blue light treatment (Fig. 1, Fig. 2). It could be that this effect is reflected in the taste of Coriander microgreens. As mentioned, in Tatsoi Purple sweetness was increased by the R60:B40 treatment (Fig. 11). The sweetness of crops is most related to their sugar content (Kozai, 2016). In this study, no significant effects on the sugar content of the microgreens were found. However, as the taste experiment was a separate experiment, it could be that in these microgreens a significant effect of the treatments on sugar content would be found, when it was analyzed. If this was indeed the case, it could be related to the fact that in the taste experiment microgreens from one batch were used instead of several batches. As a result, the effect of environmental variation in the samples was limited, which increases the chance of finding significant differences between treatments. In Mustard Frills Green firmness was found to be affected by the pre-

harvest treatments. It was found that the control group had a higher firmness compared to the pre-harvest treatments, followed by microgreens from the 440 µmol m^{-2} s^{-1} treatment (Fig. 11). It seems likely that firmness is related to the dry weight content of microgreens. However, no concrete evidence of this could be found in literature or this study. More research must be done to understand the cause of the increase in firmness that was found in this project.

The appearance of microgreens was only found to be affected in Mustard Frills Green. More blue light resulted in less purple microgreens, that had a lighter green color. On the contrary, microgreens from the control group had darker green cotyledons with more purple in them (Fig. 11). The green color in plants corresponds to the concentration of chlorophyll in the plant, while purple collaring relates to the concentration of anthocyanins. Different from this study, a study found that in cabbage and arugula blue light caused less pure green cotyledons, and darker cotyledons in red mustard, which was likely due to an increase in anthocyanins (Ying et al., 2020). Similarly, it was found that increasing the amount of blue light during cultivation in lettuce can increase anthocyanin production and create lettuce with an increasing amount of purple. This effect was only found for one of the lettuce species in the experiment (Nicole et al., 2016). In this study, blue light decreased the amount of purple in Mustard Frills Green. It is therefore likely that here the blue light treatment caused no increase in anthocyanin. Looking at research projects that have studied the biosynthetic pathways that are related to anthocyanin concentration, it becomes clear that not only blue light is important for anthocyanin concentration, but that also other colors of light play a role (Jung et al., 2021; Lobiuc et al., 2017; Meas et al., 2020). It was found that red light treatments can also increase the concentration of anthocyanins (Jung et al., 2021). This was supported by the research of Lobiuc et al. (2017) in which it was stated that anthocyanin synthesis is mediated by both red and blue light receptors. For red amaranth, an R70:B30 spectrum created the highest concentration of anthocyanin compared to white, red, and blue LED light respectively (Meas et al., 2020). This has the same amount of red light as the control treatment and a percentage of blue light in between that of the control treatment and the blue light treatment. Even though the effects of light on the concentration of anthocyanin concentration are again species-specific, this shows that it is possible to increase anthocyanin concentration with treatments that contain less blue light. In addition, it was found that in green-leaved species, green light can also increase the concentration of anthocyanin (Jung et al., 2021). Therefore, the green light that was present in the control treatment could have contributed to the higher amount of purple, and thus higher concentration of anthocyanins, in microgreens that received the control treatment. As mentioned, the green color in plants is related to the amount of chlorophyll that is present. In this study, the blue light treatment decreased the darkness of green, while the control light treatment increased it. No study could be found that analyzed the effect of light treatments on this attribute. However, studies that investigated the effect of light on the concentration of chlorophyll were done. Regarding the light spectrum, it was found that blue light drives chlorophyll production, as blue light improves the gene expression of certain genes that are involved in chlorophyll production, such as FeCH, GluTR, and MgCH (Lobiuc et al., 2017). Red light is however also important for chlorophyll production. It was found that red light leads to a reduction of 5-aminolevulinic acid which is a tetrapyrrole precursor that is required for chlorophyll synthesis. (Lobiuc et al., 2017). In addition, red light is important for the overall health of microgreens and chlorophyll production (Zhang et al., 2020). In practice it seems that mostly spectra with a lot of red light and a smaller percentage of blue light seem to increase chlorophyll production. In broccoli microgreens, a light spectrum of R80:B20 increased chlorophyll content, and in amaranth microgreens a spectrum of R70:B30 seemed to be

optimal (Meas et al., 2020; Zhang et al., 2020). These results seem to be in line with was found in this study regarding the color of the microgreens. In this project also the concentration of chlorophylls and phenols was analyzed, among which probably anthocyanin concentration. This was done in the main experiment, which was a separate experiment. No difference in chlorophyll content between the treatments was found. The concentration of several phenols was found to be influenced, however at this moment it is unclear which compounds these phenols represent. It could be that anthocyanin concentration was affected, but to investigate this the phenols must be identified. In the taste experiment a relatively small number of people participated (4-6). The people that took part in the study reported large differences between the microgreens from different treatment. It is therefore expected that more differences in taste are found when more people would have participated in the study. Accordingly, it is interesting to design a larger study on the topic. In the taste experiment also overall liking of the microgreens was tested. No significant differences between treatments were found here, which was due to strong personal preferences. This indicates that using pre-harvest treatments to influence taste and appearance of microgreens is especially useful to specifically improve certain attributes that are valued by a specific customer group. More research must be done to identify which quality attributes are valued by which customer segment for GROWx to use pre-harvest treatments optimally in this way.

Lastly, the effect of the pre-harvest treatments on yield attributes was investigated as besides quality, yield is very important for the revenue of farms. The effects of the treatments on yield attributes were studied in a broad way, in which fresh weight, dry weight, and hypocotyl length were taken into account. The treatments in this study were found not to affect these attributes, which is beneficial for the implementation of the pre-harvest treatments, as this shows that quality can be increased without loss in yield. Other research projects did show effects of pre-harvest light treatments on yield attributes when trying to increase quality, however not in all cases. When effects were present, mostly a decrease in yield attributes was found, related to stressful light conditions, such as light with a high percentage of blue or a high light intensity (Oh & Rajashekar, 2009; Ying et al., 2020). Gómez & Jiménez (2020) for instance found a decrease in fresh weight that was related to blue light for two out of three lettuce species. The third species was unaffected. In addition, Ying et al. (2020) found that in mustard, when the blue light percentage was increased from 5% to 30% during the whole growth period, hypocotyl length decreased linearly. Fresh weight was not affected in this study. Lastly, Oh & Rajashekar, (2009) found that light shocks in which high light intensity (700 µmol m^{-2} s^{-1}) was subjected to the plants for one day resulted in a decrease of fresh weight in sprouts. Based on these studies, it was hypothesized that blue light and high light treatments could negatively affect yield attributes. Fortunately, effects like this were not found in this study (Fig. 12). Yet, as batch was found to affect the yield attributes, it could be that effects of the treatments on fresh weight, dry weight, and hypocotyl weight are found when environmental variation is limited (Appendix G). However, it seems that pre-harvest treatments, with a short application period, do not strongly affect yield attributes. If an effect of pre-harvest treatments is therefore present, no large effect is expected. In addition, this study shows that quality attributes are affected more severely than yield attributes. Potential effects on yield attributes are, as a consequence, likely not limiting.

Some limitations are present in this study, which might have influenced the results of this study. As already discussed, the largest limitation of this study is the environmental variation that was present in the growing cell during the experiment. The observed variations in environment were caused by errors related to water gift and fluctuations in temperature and humidity in the growing cell (Appendix

B). Differences in water gift were caused by regular errors of the robots that provided the gutters with microgreens with water during the experiment. These errors resulted in less water being given to the plants or the delay of water gift, which sometimes resulted in the death of microgreens. During the experiment, the effect of these variations was minimized by excluding dead plants from analysis, compensating for dead plants in fresh weight measurements (section 2.3), and by using non-damaged pieces of substrate with microgreens when this was possible. Still, an effect of environmental variation was thought to be present. To study the effect of the variation in the environment that remained, the effect of batch was analyzed. Significant differences in yield, shelf life, and nutritional value were found when different batches of microgreens were compared (Appendix E, F, G). The differences in shelf life can almost completely be explained by the different treatments that were analyzed in the batches (Appendix F). For yield and nutritional value, this is not the case (Appendix E, G). For these attributes the effect of batch was found to be larger than the effect of the pre-harvest treatments, resulting in increased uncertainty surrounding the results of this study. In addition, the effect of batch likely overshadows the effect of the treatments, which means that more severe effects of the treatments potentially are found in a more stable environment. As the taste experiment was done with microgreens from one batch, the influence of environmental variation is limited here. Besides the effect of batch, a limitation of this study is that layers and sublayers in the vertical farm could not be separated by reflective plastic, which was related to the movement of the robot. The influence of the light environment by lights on another layer or sublayer is therefore not completely ruled out. However, efforts were made to limit such effects, for instance by creating distance between the sublayers where treatments were supplied to the plants. Effects are therefore likely minimal.

In addition to studying the effects of pre-harvest treatments on several quality and yield attributes, effort has been made to develop a tool that can make extensive research projects on the optimal environment of microgreens cheaper and faster, by the development of PLS regression models that can predict the concentration of compounds based on VIS-NIR spectrometry data. Such models could be implemented in the automated vertical farming system of GROWx and are especially interesting as many studies still have to be done to fully optimize the growth environment for each species that is cultured. The models were developed with the HPLC, LCMS, and Dionex data of Coriander, Mustard Frills Green, and Tatsoi Purple, which was also used to get insight into the effect of pre-harvest treatments on the concentration of metabolites. The PLS models that were developed for the studied metabolites during the pilot seemed to have a high potential (Fig. 13). The accuracy of the models was low to medium, but an increase in accuracy was expected when more data was analyzed. However, during the main experiment, it became clear that accuracy did not increase as much as was needed to obtain useful models (Fig. 14). It is thought that the low accuracy of these models could be due to experimental error in the HPLC-PDA measurements, which decreased the accuracy of the concentrations of the metabolites that were obtained. Relatively large differences in values for the internal standard were namely observed, for the repeated internal standard measurements (S. Hageraats, *personal communications*, 14-7-2021). At this moment the models that were developed are not useful, but due to the immense potential that PLS regression models based on spectrometry offer, it remains interesting to put effort towards their development in the future. Earlier research showed that it is possible to develop similar PLS models with HPLC data (Renner & Fritz, 2020). Differences in experimental methods between the two studies must be further examined when GROWx wants to continue with the development of PLS regression models based on spectrometry. Additionally, it is interesting to look into the use of other experimental methods to develop the PLS regression models.

It must be noted that the development of such models can be costly. However, in the long run, they are expected to pay off.

4. Conclusion

Concluding, this study provided insight into the potential application of pre-harvest light treatments in a commercial vertical farm. It has been shown that nutritional value, shelf life, and taste can be influenced by different pre-harvest light treatments, without influencing the yield. This provides great possibilities regarding the improvement of microgreen quality within GROWx's vertical farming system. When it is exactly known how pre-harvest treatments can influence each quality attribute, pre-harvest treatments can be used to specifically improve the microgreen varieties in a way that is valued for that specific species. In regular horticulture, this would be impossible but in GROWx's vertical farming system targeted pre-harvest treatments have real potential. Benefits of specific pre-harvest treatments include the production of microgreens with a specific nutritional function or specific taste. Both can attract new customers and bind existing ones to the company. Lastly, the treatments could be used to increase the shelf life of the microgreens, which can increase revenue as well. It must be noted that this study shows that each quality attribute responds differently to the light treatments. Using pre-harvest treatments to specifically optimize one, or a set of, quality attributes has, therefore, more potential than using the treatments to optimize overall microgreen quality. Lastly, in this study, it has been shown that pre-harvest treatments affect each species differently, which is likely due to species-specific biosynthetic pathways for metabolites, and different optimum environmental conditions. This highlights that for the application of pre-harvest treatments across the farm, more research is needed.

5. Future studies

As GROWx cultivates many different microgreens species, additional studies must be done before pre-harvest treatment can be implemented within GROWx. The following process is suggested: first, it would be interesting to perform consumer research to determine which specific quality attributes add value for the consumer. Some information about this is already known, but GROWx cultivates rare microgreens for specific consumers. It is therefore interesting to spend some time researching which attributes (e.g. specific tastes) are valued for each microgreen species by the customer. Afterward, it is suggested that research is done on how pre-harvest treatments can improve these valued quality attributes. This can be done the conventional way: a series of costly experiments for each species cultured, in which the effects of pre-harvest treatments on the quality attributes are tested. Or, which is advised, efforts can be made to further develop the PLS regression models described in this study and work towards using this technology to optimize the vertical farming system in a less costly way. As the development of the PLS regression models was not successful in this study, it is advised to look into ways to improve or change the experimental methods first. When the models are successfully developed, they can be used for research on the use of pre-harvest treatments, but also research on other aspects of the environment. After development, models can immediately be used for research related to nutritional value. For research on other quality attributes, first correlations must be found between the quality attributes and compounds in the plant. When clear correlations between quality attributes and identified compounds, whose concentration can be accurately estimated, are found, the full potential of the tool is reached. The effect of pre-harvest treatments on each of the species cultured can then be studied in a broad, time-efficient, and cheap way. The further development of

the model can be done similarly to this research project. Studies on the effect of an aspect on the environment on the plant can be done, and the samples created can be used for the development of the model. Optimization of the cell and optimization of the research process can then go hand in hand. Following the large effect of batch that was found in this study, it is recommended to continue this process when the stability of the research cell is improved. In addition, it is recommended to continue this research when GROWx is further established. Research on the use of pre-harvest light to increase quality is expensive, as is the development of models that predict the concentration of plant compounds that can eventually make the process cheaper and faster. As each species is affected differently, knowledge that is generated on this topic cannot be implemented directly across the farms. Therefore, more basic research projects regarding the development of proper environmental conditions should be given priority.

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Appendix A – Overview set-up in climate cell

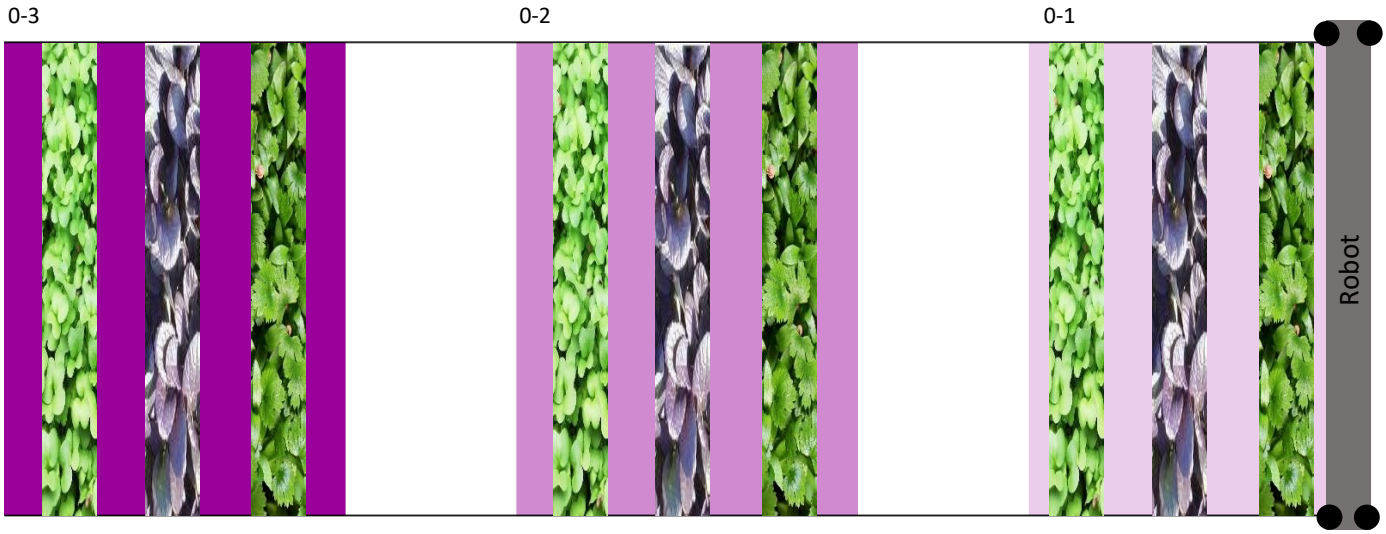


Fig. A1: Overview of set-up climate cell that was used in this study. Here, the pre-harvest layer is shown. In between treatments groups no reflective plastic was present, as this was prohibited by the movement of the robot. To minimize the influence of treatments on eachother distance between groups was created.

Appendix B – Temperature and humidity in climate cell

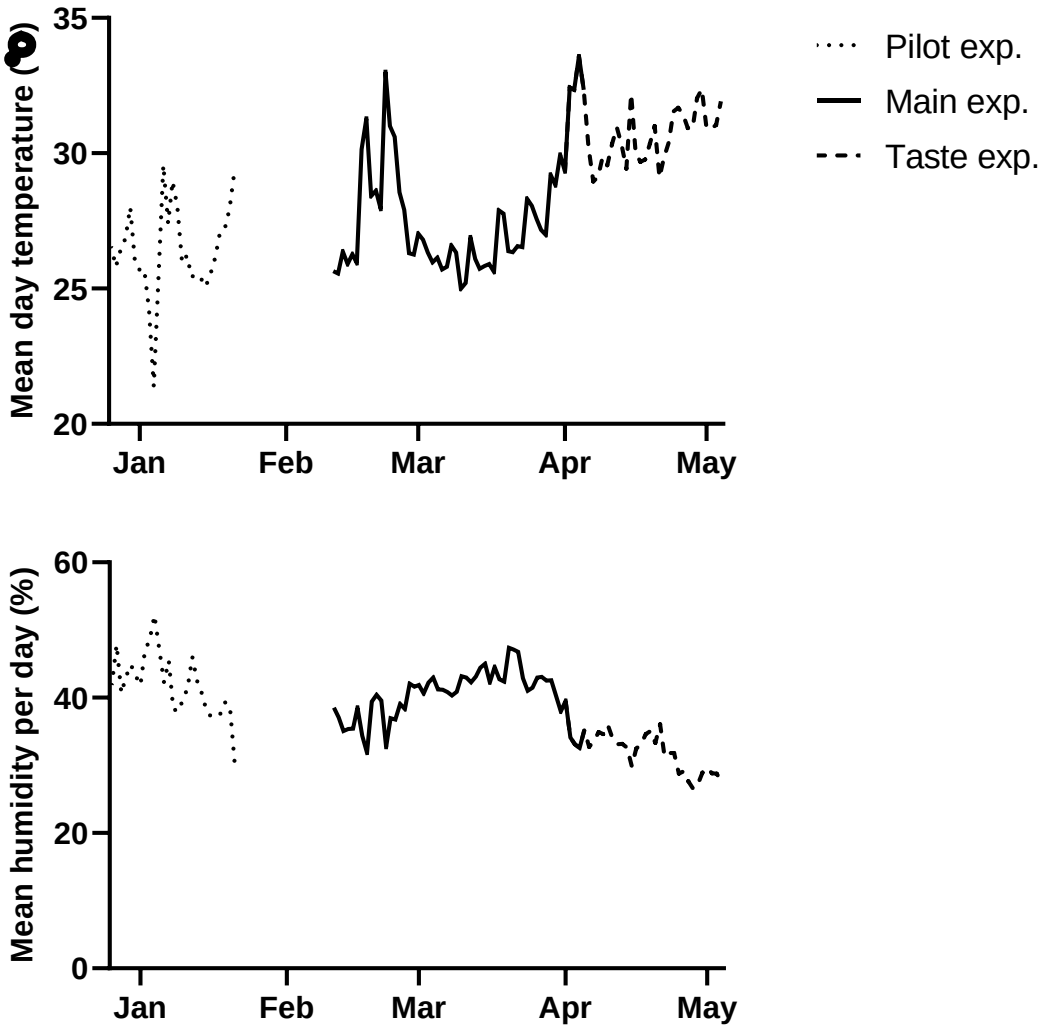


Fig. B1: Variation in mean day temperature and mean humidity during the experimental study. Sensory data of the robot is shown here and was averaged for the vertical farming layers that were used in the experiment. The effect of this variation + the variation in water gift (data not shown) is thought to cause sig. differences between batches. Batch 1 (26-12 until 14-1), Batch 2 (2-1 until 21-1), Batch 3 (11-2 until 2-3), Batch 4 (18-2 until 9-3), Batch 5 (25-2 until 16-3), Batch 6 (4-5 until 23-3), Batch 7 (11-3 until 30-3), Batch 8 (18-3 until 6-4).

Appendix C – Nutrient mix

NUTRIENT SOLUTION COMPOSITION Standard Mix

Crop and stage	Standard Mix : Stage: : inserted values
Volume of stock tanks (L):	3
Dilution ratio	1: 100
Set-point pH:	5,7
Target EC (dS/m):	2,35
Expected EC (dS/m)	2,40

Ionic ratios (expressed in milliequivalent)

N/K	2,70		
NH_4/NO_3	0,08		
K:Ca: Mg	#DIV/0!	#DIV/0!	#DIV/0!

Irrigation water	?	(uM for Fe, B, Cu, Zn, Mn, Mo; mM for other ions)														
EC (mS/cm)	HCO^{3-}	$N\text{-}NO_3$	$N\text{-}NH_4$	P	K	Ca	Mg	Na	$S\text{-}SO_4$	Cl	Fe	B	Cu	Zn	Mn	Mo
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Recipes	0,00	0,0	0,0	0,0	Fertilizers and acids	0,0	0,0
ppm	*177*	*4*	*2*	*1*	*6*	*40*	*7*	*55*	*5*	*67*	*0,02*	*0,05*	*0,04*	*0,03*	*0,01*	*0,05*
OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK

Fig. C1: Standard nutrient mix of GROWx. In this experiment, during the growth and pre-harvest phase, plants received a received a diluted (2x) version of the standard nutrient mix presented here.

Appendix D – Scoresheet Coriander

CORIANDER: 536, 412, 786, 217

Scoresheet **Naam:**

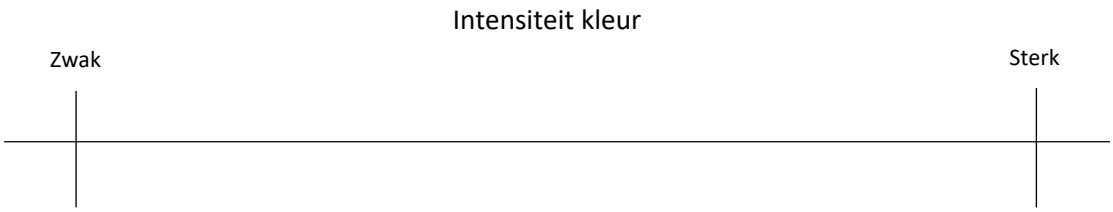
Ik houdt wel / Ik houdt niet van Coriander (omcirkel welke voor jou geldt)

- Neem je tijd
- Drink tussendoor water en eet wat crackers
- Eerst kijken dan ruiken en dan proeven
- Gebruik een streepje met twee cijfers erboven om aan te geven dat de twee soorten hetzelfde smaken bij die vraag.
- Je mag ook het stuk na de verticale lijn gebruiken met beschrijving gebruiken. VB. streepje rechts van verticale streep met ‘’Heel bitter”

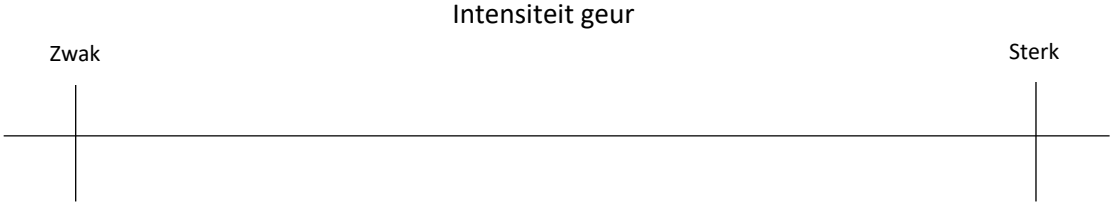
Zet een verticaal streepje op de lijn met het driecijferige nummer erboven.

Voorbeeld

Uiterlijk

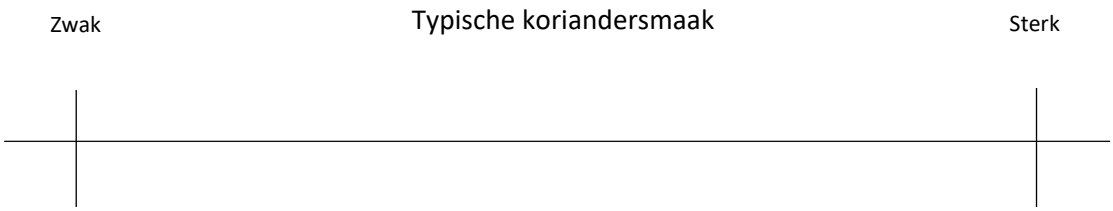


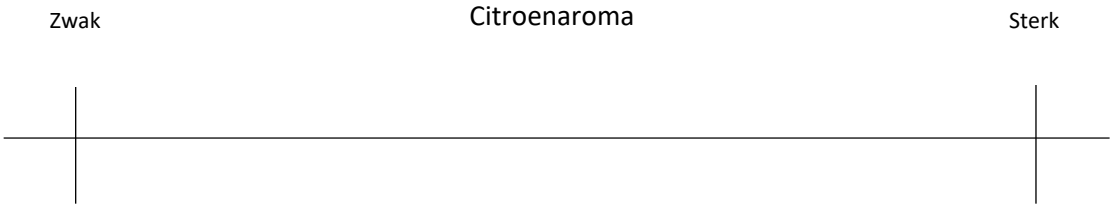
Geur



Structuur

Smaak





Zout

Niet zoutig — Erg zoutig

Bitterheid (proeven met neus dicht)

Zwak — Sterk

Overall smaakbeleving

Vlak — Intens

Overal liking

Weinig waardering — Veel waardering

Reden dat de een lekkerder is dan de ander:

Opmerkingen

536:

412:

786:

217:

Algemeen:

Appendix E – Nutritional compounds as affected by batch (PCA)

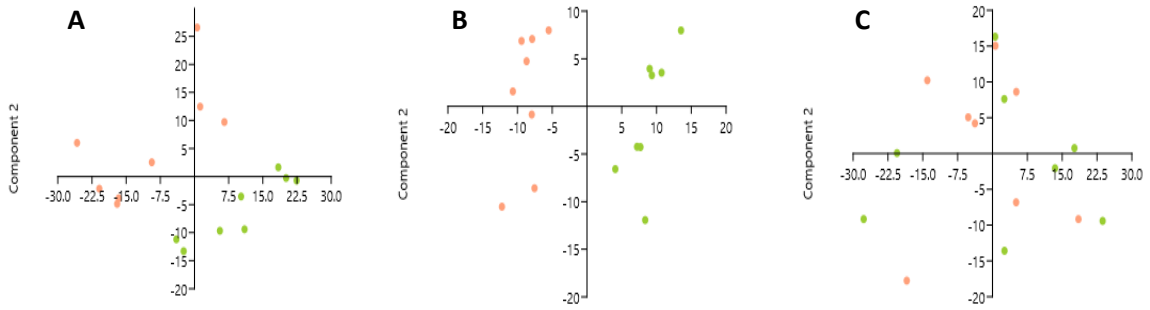


Fig E1: Clear separation of batches in PCA with all measured metabolites in Coriander (A), Mustard Frills Green (B). In Tatsoi Purple (C) no clear separation is present. The colored dots represent the different batches. Salmon: batch 1 (26-12 until 14-1), green: batch 2 (2-1 until 21-1). Data analyzed include chlorophyll A and B, carotenoids, phenols, α-tocopherol, glucose, sucrose, fructose, ascorbic acid and glucosinolates. Data was generated with LCMS, HLPC-PDA and a Dionex after which log transformation took place. PCA is based on correlation. Four replicas per treatment were used. In coriander (A) PC1 explains 42.0% of the variance and PC2 explains 19.5%. In mustard (B) PC1 explains 35.6% of the variance and PC2 explains 19.0%. In tatsoi (C) PC1 explains 35.0 % of the variance and PC2 explains 17.1%.

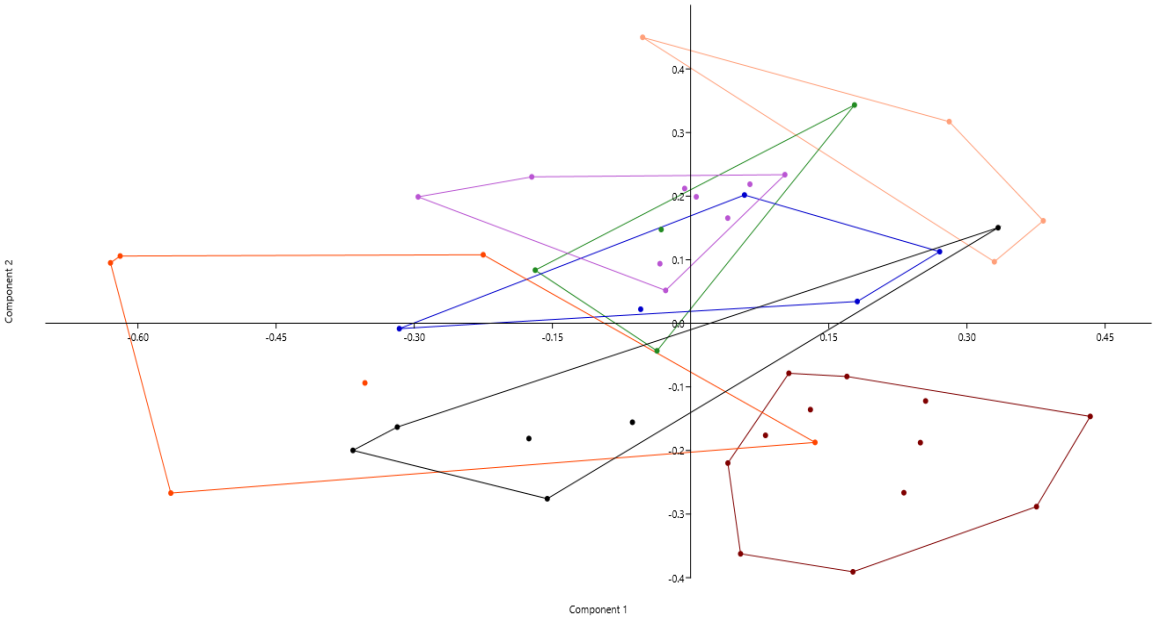


Fig E2: Separation of batches was found when PCA was done for the carotenoids, chlorophylls and tocopherols that were analyzed in the main experiment, as influenced by pre-harvest treatments (5 days), in Coriander. Log transformed data was used. n=6-10 for control, R90:B10, 440 µmol m^{-2} s^{-1}, R60:B40 treatment. n=4 for 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50 treatment. Light intensity control treatment: 240 µmol m^{-2} s^{-1}. Light spectrum control treatment: R70:G10:B20. Treatments are named according to their deviation from the control treatment. PCA is based on correlation. The different color dots in represent the batches. Salmon: batch 1 (26-12 until 14-1), red: batch 3 (11-2 until 2-3), Blue: batch 4 (18-2 until 9-3), green: batch 5 (25-2 until 16-3), purple: batch 6 (4-5 until 23-3), orange: batch 7 (11-3 until 30-3), black: batch 8 (18-3 until 6-4). In batch 1 till 6 the 440 µmol m^{-2} s^{-1} , the R60:B40, the R90:B10, and the control treatment have been tested. In batch 7 and 8 the 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1} and R90:B10 treatment have been tested. n=6-10 for control, R90:B10, 440 µmol m^{-2} s^{-1}, R60:B40 treatment. n=4 for 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50 treatment. PC1 explains

Appendix F – Shelf life as influenced by batch

A significant difference in shelf life was found for Coriander and Tatsoi Purple microgreens of different batches (p=0.000, 0.000) (Fig. F1). In Coriander significant differences were found between batch 3, 4, 5 and batch 6, 7, 8. Treatment 5 differed significantly from all treatments (p=0.000). In Tatsoi Purple batch 5 differed significantly from treatment 7 (p=0.000) and 8 (p=0.001). Treatment 6 differed significantly from treatment 7 (p=0.010). It must be noted that in batch 7 and 8 a different set of treatments was analyzed. In this case, the significant differences found are largely caused by this difference.

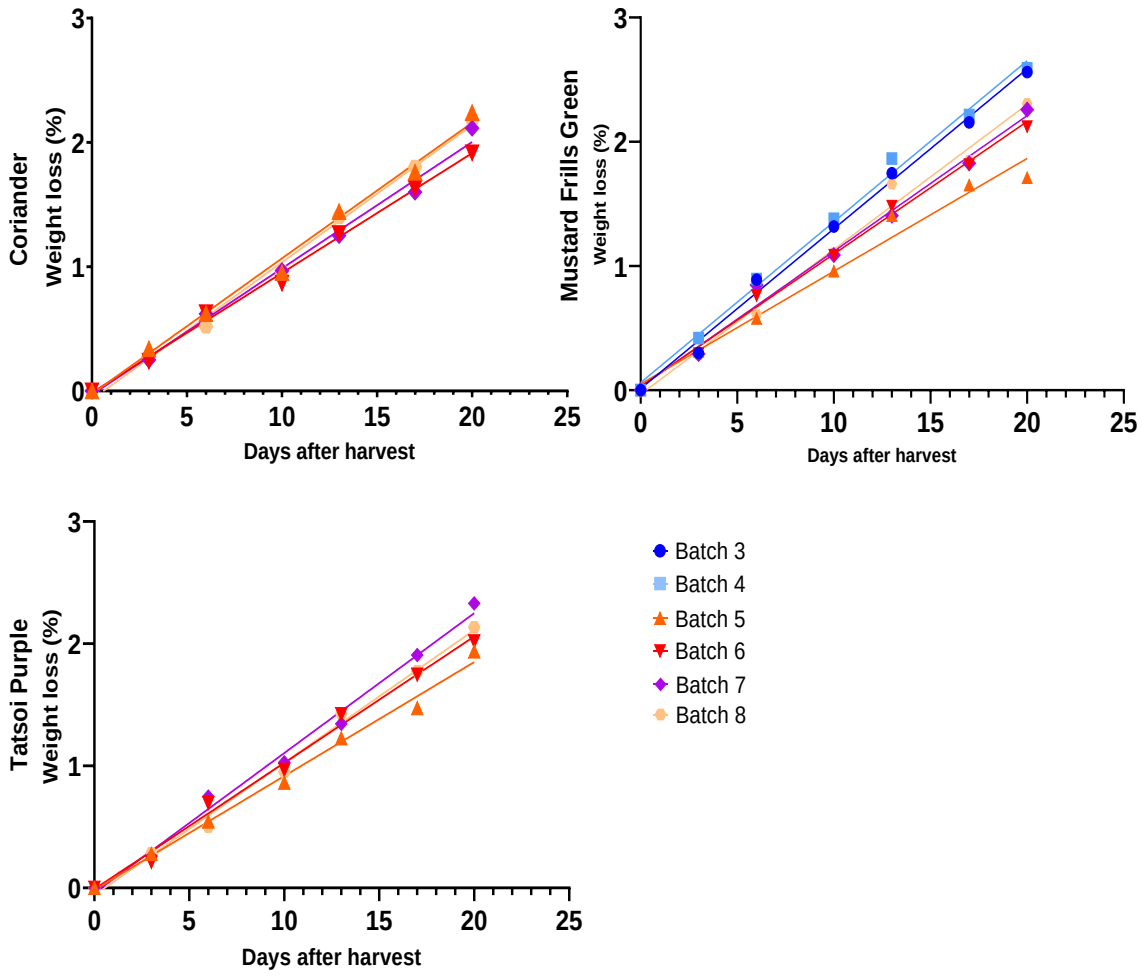


Fig. F1: Shelf life is influenced by batch, but this is largely caused by variation in treatments in batches. Weight loss after harvest was measured by weighing stored boxes of microgreens twice a week and calculating weight loss with the formula (Weight t, t / Weight t, 0) * 100%. An ANCOVA model was used to find significant differences in slope., which represents the speed at which the microgreens go bad. Storage took place in a dark room with a temperature of 10 °C. Batch 3 (11-2 until 2-3, n=12), Batch 4 (18-2 until 9-3, n=6), Batch 5 (25-2 until 16-3, n=4-8), Batch 6 (4-5 until 23-3, n=8-9), Batch 7 (11-3 until 30-3, n=5-6), Batch 8 (18-3 until 6-4, n=6). In batch 3 till 6 the 440 µmol m−2 s−1 , the R60:B40, the R90:B10, and the control treatment have been tested. In batch 7 and 8 the 140 µmol m−2 s−1, 640 µmol m−2 s−1 and R90:B10 treatment have been tested. All treatments took place for 5 days before harvest. Light intensity control group: 240 µmol m^{-2} s^{-1} . Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control.

Appendix G – Yield attributes as influenced by batch

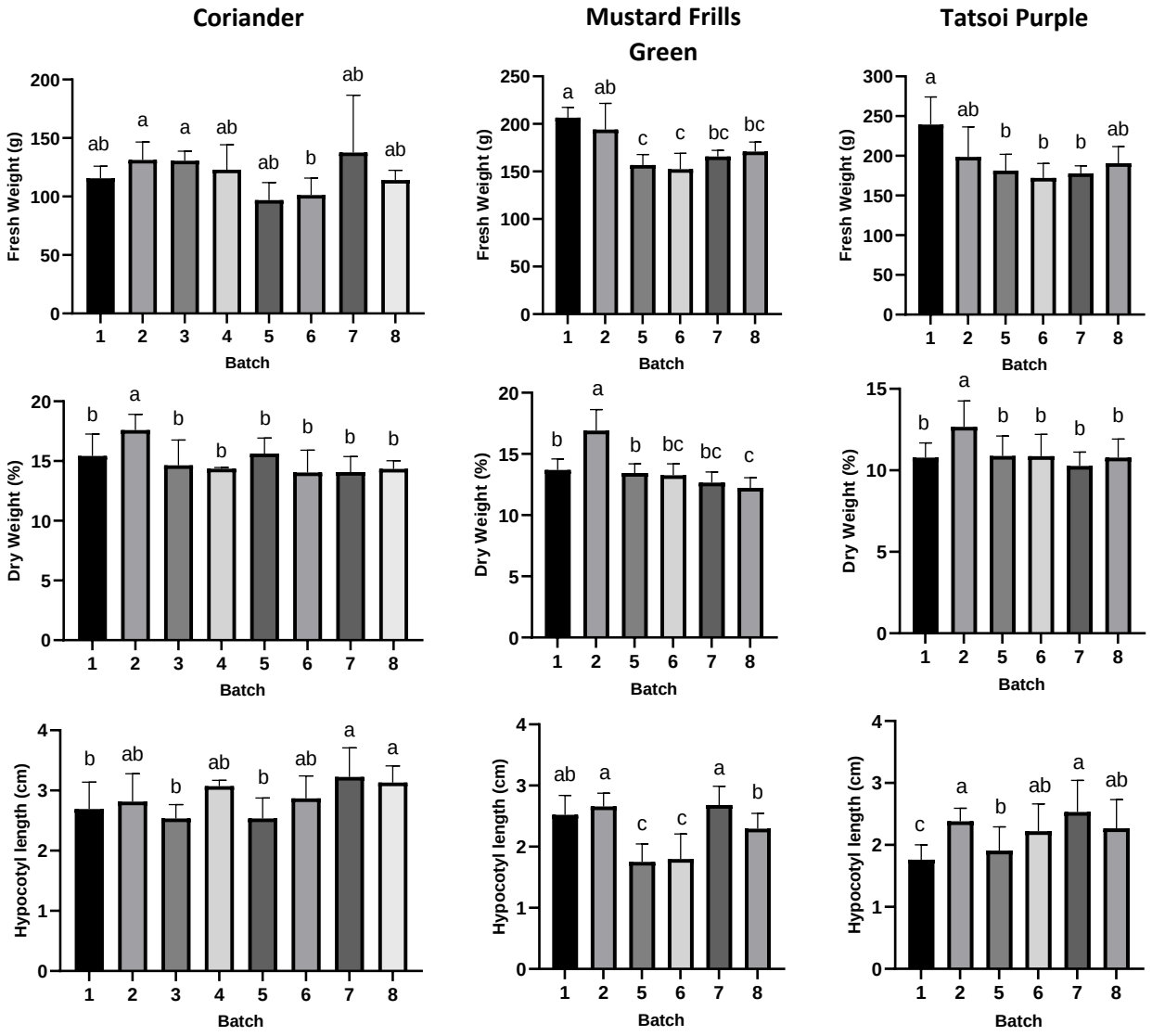


Fig. G1: Batch affects fresh weight (g), dry weight (%) and hypocotyl length (cm) in Coriander, Mustard Frills Green and Tatsoi Purple. Fresh weight was measured by weighing all microgreens from a gutter directly after harvest. Dry weight was measured by weighing the microgreens before and after they were dried in an oven for 24-48h at 105 °C. With this data the dry weight % was calculated ((dry weight/ fresh weight) *100%). For hypocotyl length the length from the beginning of the stem till the hypocotyl was measured with a ruler directly after harvest. The length of five microgreens per gutter was averaged an used as one experimental unit. Statistical analysis was done with One -way ANOVA and Brown-Forsythe ($p \leq 0.05$). Batch 1 (26-12 until 14-1, n=8), Batch 2 (2-1 until 21-1, n=8), Batch 3 (11-2 until 2-3, n=12), Batch 4 (18-2 until 9-3, n=6), Batch 5 (25-2 until 16-3, n=4-8), Batch 6 (4-5 until 23-3, n=8-9), Batch 7 (11-3 until 30-3, n=5-6), Batch 8 (18-3 until 6-4, n=6). In batch 1 till 6 the 440 µmol m^{-2} s^{-1}, the R60:B40 and the R90:B10 treatment have been tested. In batch 7 and 8 the 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1} and R90:B10 treatment have been tested. Light intensity control group: 240 µmol m^{-2} s^{-1}. All treatments took place for 5 days before harvest. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control. The letters on top of the bars indicate sig. differences.

Appendix H – Detailed results of PLS regression modeling per compound

In the following section, the results of the development of PLS regression models that predict the concentration of interesting compounds in plants based on VIS-NIR spectrometry are discussed in detail. Results are discussed per compound. For most compounds, only models were developed during the pilot. For chlorophyll, carotenoids and tocopherols models were developed during both the pilot and main experiment. During the main experiment, more samples were analyzed to get a better idea of the potential of the development of models with the described experimental methods.

5.6.1. Sugars

Modeling of the glucose, fructose, and sucrose data resulted in a high R^2 when all species were analyzed together (Fig. H1). When a model is made for each of the species separately, the situation is different. For most sugars and individual species, the R^2 is low. Looking at the scatter plot it becomes clear why. The variation in sugar content between species is quite large, but within species, it is quite low. This makes that the PLS regression model in its current form acts mostly to discriminate between the species, rather than correctly predict the variation in sugar content within one species. Therefore, the R^2 values listed on top reflect mostly species classification and only in part sugar content regression accuracy. Accordingly, at this point, the model cannot be used to accurately predict the concentration of sugars within species, as was intended. This is unfortunate as such models could play a role in automated experiments regarding taste.

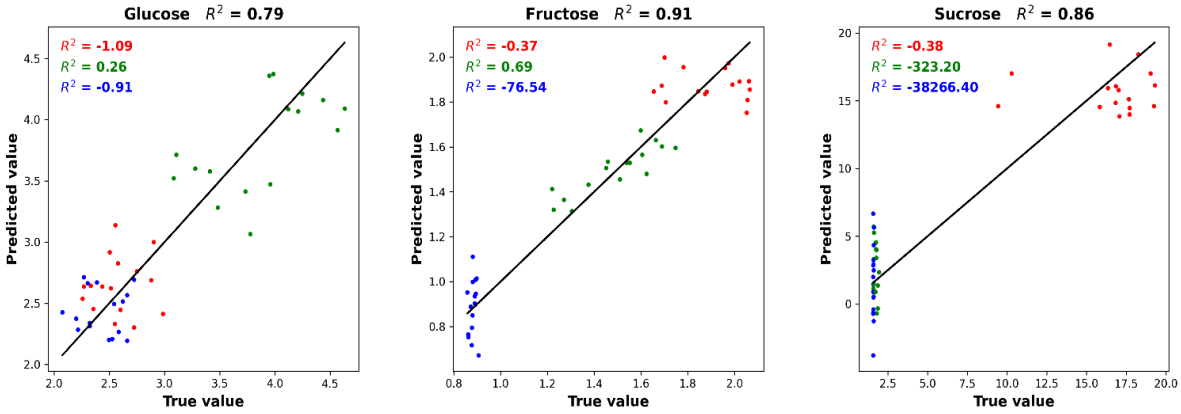


Fig H1. PLS regression models shows potential to discriminate between species (high R^2) based on sugar content, but cannot be used to predict the concentration of sugars within species (low R^2) at this point. PLS regression model of predicted value using VIS-NIR spectrometry versus true value is presented for Coriander, Mustard Frills Green and Tatsoi Purple. A Dionex was used to obtain data on glucose, fructose and sucrose concentration, as affected by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40). Red represents Coriander, green represents Mustard Frills Green and blue represents Tatsoi Purple microgreens. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

5.6.2. Ascorbic acid

Even though for ascorbic acid the measurements are quite evenly distributed along the regression line, the R^2, based on analysis of the species together, is quite low (Fig. H2). It is anticipated that the R^2 increases when more samples are used to develop the model, especially as the HPLC-PDA measurements that were done for ascorbic acid were afterwards quantified with a calibration curve and therefore more accurate. However, this remains to be studied. In the case that this indeed leads to an increased R^2, the model could be quite useful, as it would be interesting to have a tool that can

quickly give an indication of vitamin C levels across species. Analysis of individual species with this model is not useful at this point, but potentially could also be improved with more samples and acquired measurements.

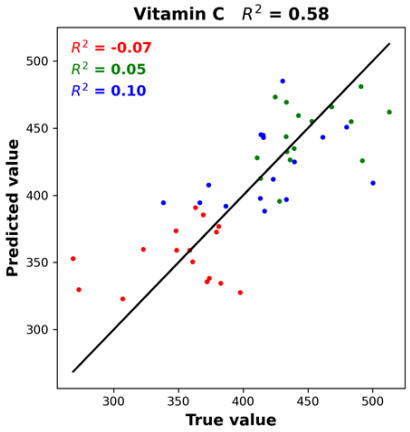


Fig H2. PLS regression model cannot accurately predict ascorbic acid content based on VIS-NIR spectrometry in Coriander (red), Mustard Frills Green (green) and Tatsoi Purple (blue) at this point. Ascorbic acid concentrations, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), were analyzed with HPLC-PDA, and were quantified with a calibration curve. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

3.5.3. Glucosinolates

To get an idea about the potential use of PLS regression modeling to predict the concentration of glucosinolates, a few glucosinolates that were present in relatively high amounts in the microgreens were chosen for the development of models. It was found that the R^2 values of the PLS regression model, for all species together, are medium to low (Fig. H3). The R^2 for each species individually is even lower, and most have negative values. This is caused by the low variation in glucosinolates within most species. Therefore, the potential of predicting the concentration of glucosinolates with PLS regression modeling based VIS-NIR spectrometry seems low.

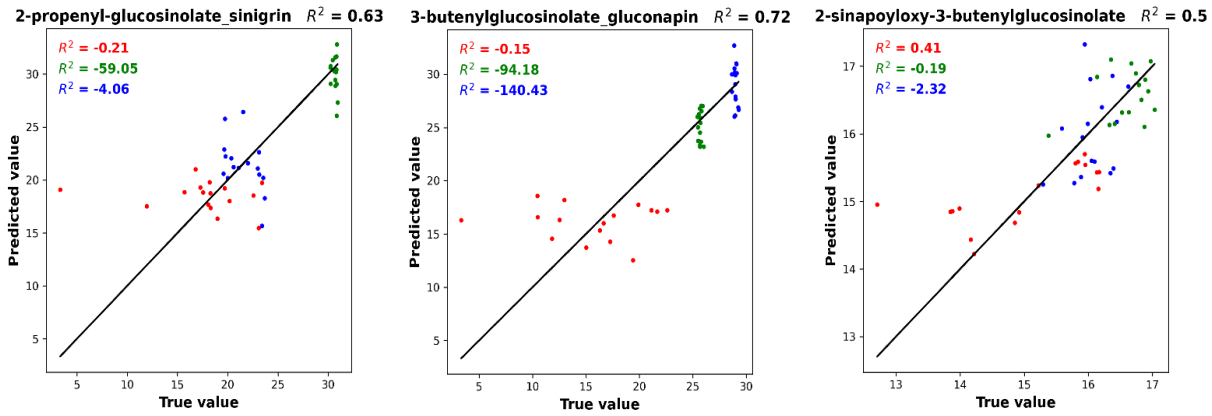


Fig H3. PLS regression model cannot accurately predict the concentration of glucosinolates based on VIS-NIR spectrometry in Coriander (red), Mustard Frills Green (green) and Tatsoi Purple (blue) at this point. Glucosinolate concentrations, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), were analyzed with LCMS. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

3.5.4. Phenols

In the pilot, a large number of phenols (>1000) were detected using LCMS. To get insight into the use of PLS regression models based on VIS-NIR spectrometry, PLS regression models were trained on all phenols. This mostly yielded very poorly performing PLS regression models. The best performing model

was trained on the peak intensities of a phenol here labeled as #772. Figure H4 shows that the prediction of the concentration of this specific phenol was not successful when individual species were analyzed. This was expected, as individual phenols are present in low amounts in the plant, which makes predicting the compound based on VIS-NIR reflection spectra quite difficult. The model was better at predicting the concentration of this phenol when all species were taken together. As phenols are present in low amounts in the plant, it is thought that the relatively high R^2 is caused by a correlation of these phenols with other compounds in the plant that are present in a higher concentration. However, it must also be stressed that due to the differences in the concentration of this phenol between different species, part of the predictive capability of this model is due to its ability to classify the species, instead of its ability to predict the concentration of this particular phenol.

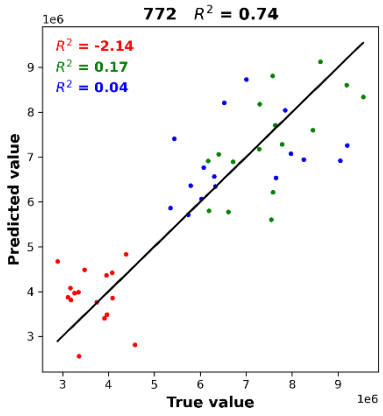


Fig H4. PLS regression model moderately predicts the concentration of glucosinolates based on VIS-NIR spectrometry when species are analyzed together (medium R^2), but is not able to accurately predict the concentration of glucosinolates within species at this point (low R^2). Concentration of phenol #772, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), was analyzed with LCMS. Red represents Coriander, green represents Mustard Frills Green and blue represents Tatsoi Purple microgreens. Light intensity control group: 240 µmol m^{-2} s^{-1} . Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

3.5.6 Carotenoids

Carotenoid concentrations were analyzed with HPLC-PDA in both the pilot and the main experiment. In the pilot, it was found that compared to other compounds, carotenoids could be relatively accurately predicted with a PLS regression model based on VIS-NIR spectrometry. For beta-carotene, which is used as an example here, this is true for both the model based on all three species and for the model based on Coriander and Mustard Frills Green individually (Fig. H5). As the results of PLS regression modeling for carotenoids showed potential, the models were further developed in the main experiment. In the main experiment, only models for Coriander were developed. This time, a lot more repetitions were used, five times as many as in the pilot, as a lot of data is needed to get insight into the potential of the model. In the pilot, only a general idea about the use of the models was generated, with many uncertainties. In the main experiment for carotenoids, PLS regression modeling based on VIS-NIR yielded low R^2 (Fig. H6). The development of models to predict carotenoids in the way that was done in this study is therefore unsuccessful. It is thought that this is due to the accuracy of the HPLC-PDA method that was used in this study to measure the carotenoid concentrations, which now seems insufficient for the development of a model.

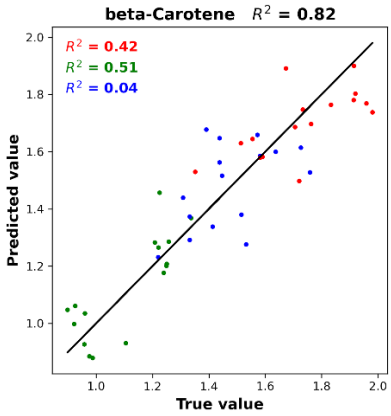


Fig H5. Example of a PLS regression model based on VIS-NIR for carotenoids that is able to predict the concentration of the compound, in this case beta-carotene, based on VIS-NIR spectrometry when species are analyzed together (high R^2). In this case, the model can also relatively accurately predict the concentration of beta-Carotnene within Coriander and Mustard Frills Green (medium R^2). The concentrations of carotenoids, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), were analyzed with HPLC-PDA. Light intensity control group: 240 µmol m^{-2} s^{-1}. Red represents Coriander, green represents Mustard Frills Green and blue represents Tatsoi Purple microgreens.Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

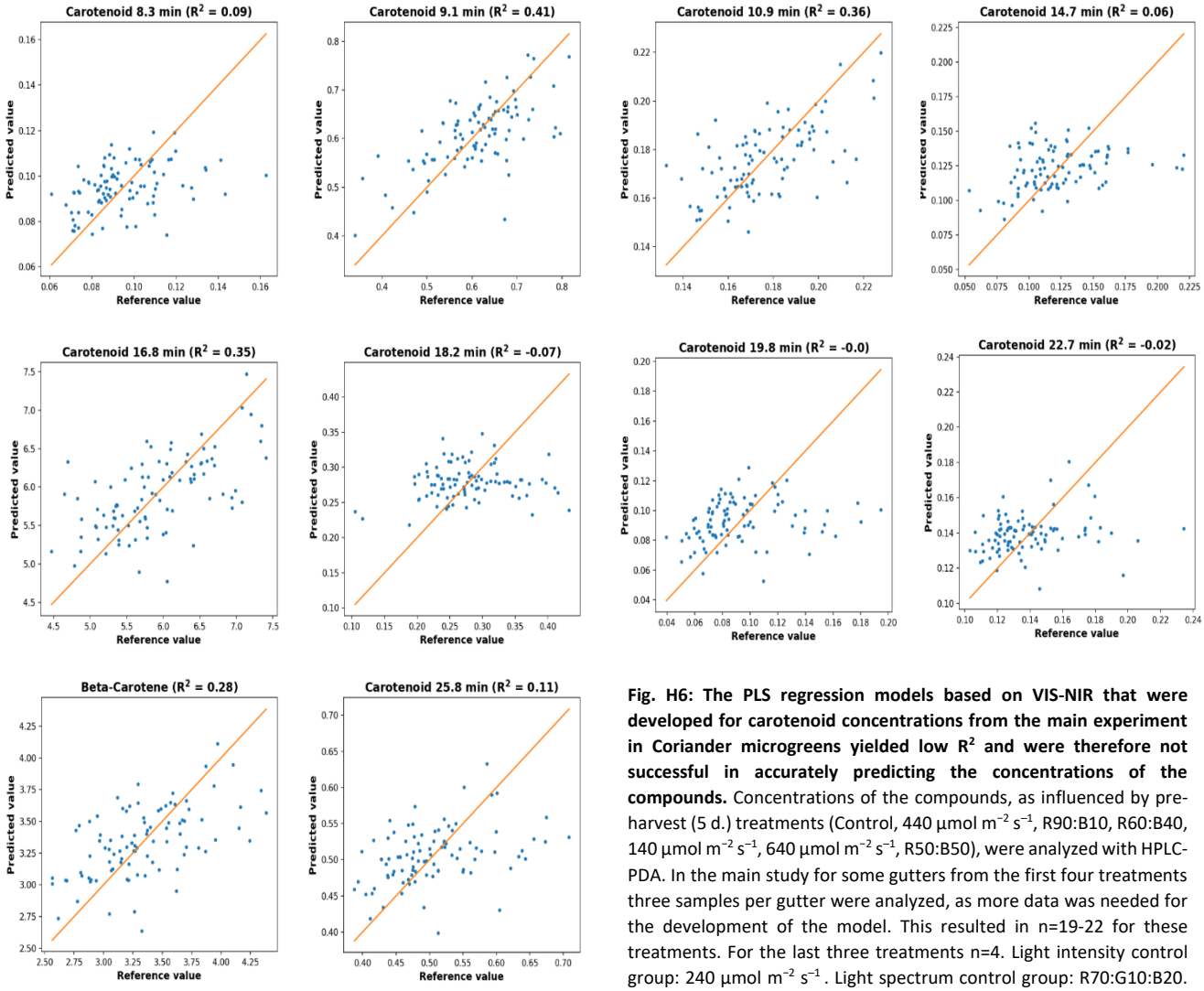


Fig. H6: The PLS regression models based on VIS-NIR that were developed for carotenoid concentrations from the main experiment in Coriander microgreens yielded low R^2 and were therefore not successful in accurately predicting the concentrations of the compounds. Concentrations of the compounds, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40, 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50), were analyzed with HPLC-PDA. In the main study for some gutters from the first four treatments three samples per gutter were analyzed, as more data was needed for the development of the model. This resulted in n=19-22 for these treatments. For the last three treatments n=4. Light intensity control group: 240 µmol m^{-2} s^{-1}. Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group.

3.5.7 Chlorophylls

Chlorophylls were also analyzed in the pilot and main experiment with HPLC-PDA. Similarly, as for the carotenoids, development of the model showed potential for chlorophylls during the pilot (Fig. H7), but during the main experiment, it became clear that with the current methods no useful model can be developed for the compound (Fig. H7). Here also, it is thought that this is due to the accuracy of the HPLC-PDA method that was used in this study to measure the carotenoid concentrations, which now seems insufficient for the development of a model.

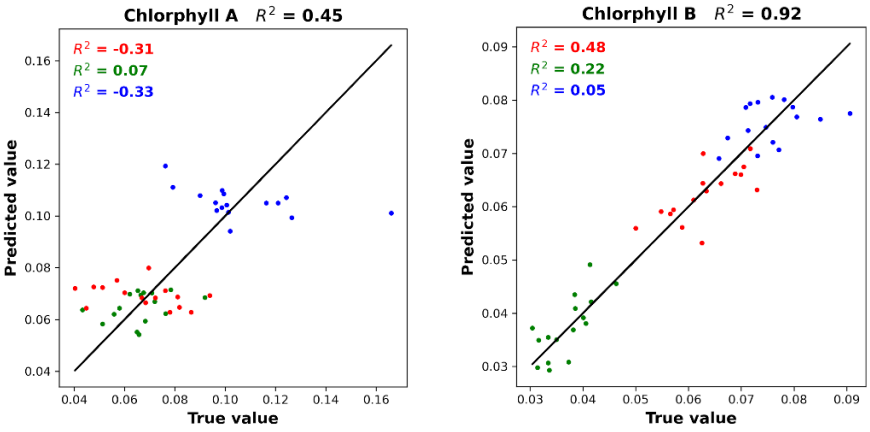


Fig. H7: PLS regression models and B are that is able to predict the concentration of the compound, in this case beta-carotene, based on VIS-NIR spectrometry when species are analyzed together (high R^2). The concentrations of cholorphylls, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), were analyzed with HPLC-PDA. Light intensity control group: 240 µmol m^{-2} s^{-1}. Red represents Coriander, green represents Mustard Frills Green and blue represents Tatsoi Purple microgreens.Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

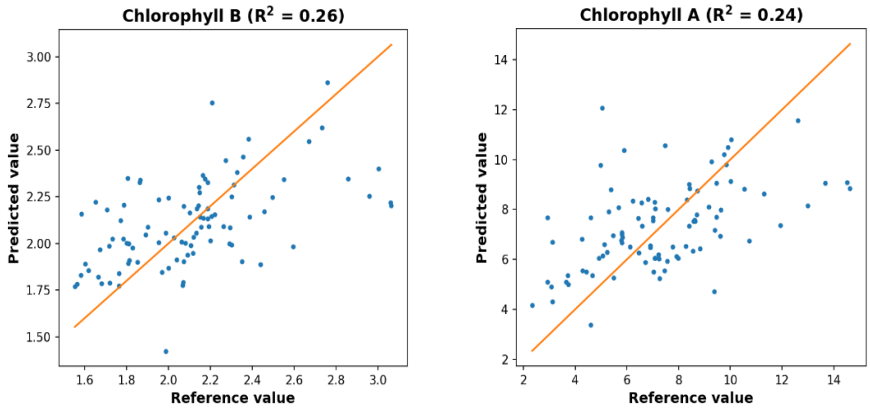


Fig. H8: The PLS regression models based on VIS-NIR that were developed for chlorophyl concentrations from the main experiment in Coriander microgreens yielded low R^2 and were therefore not successful in accurately predicting the concentrations of the compounds. Concentrations of the compounds, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40, 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50), were analyzed with HPLC-PDA. In the main study for some gutters from the first four treatments three samples per gutter were analyzed, as more data was needed for the development of the model. This resulted in n=19-22 for these treatments. For the last three treatments n=4. Light intensity control group: 240 µmol m^{-2} s^{-1} . Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group.

3.5.8 Tocopherols

During the pilot, the PLS model based on VIS-NIR spectrometry that was developed for α-tocopherol was most successful, compared to the models for other compounds. A high R^2 when all species were analyzed together was found in addition to high R^2 values for models for Mustard Frills Green and Tatsoi Purple specifically (Fig. H9). In Coriander microgreens the R^2 was low. In the main experiment, Coriander microgreens were further studied for carotenoids, chlorophyll, and tocopherols with HPLC-PDA. This data was also used for the development of PLS regression models. Unfortunately, in the main experiment, the R^2 of the PLS regression model for Coriander remained low.

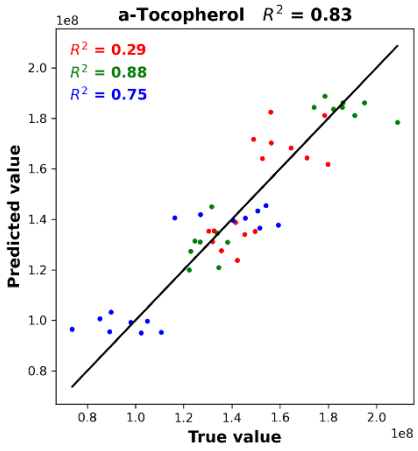


Fig H9. PLS regression model based on VIS-NIR for tocopherol is able to accurately predict the concentrations of the compounds across species (high R^2) and within Mustard Frills Green and Tatsoi Purple microgreens (high R^2). The concentrations of tocopherol, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40), were analyzed with HPLC-PDA. Light intensity control group: 240 µmol m^{-2} s^{-1}. Red represents Coriander, green represents Mustard Frills Green and blue represents Tatsoi Purple microgreens.Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group. Four replicas per treatment are used.

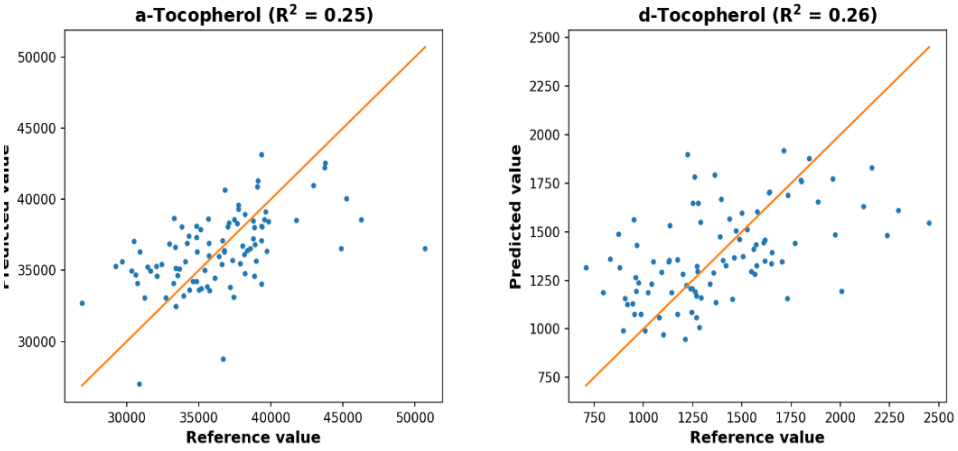


Fig. H10: The PLS regression models based on VIS-NIR that were developed for tocopherol concentrations from the main experiment in Coriander microgreens yielded low R^2 and were therefore not successful in accurately predicting the concentrations of the compounds. Concentrations of the compounds, as influenced by pre-harvest (5 d.) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40, 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50), were analyzed with HPLC-PDA. In the main study for some gutters from the first four treatments three samples per gutter were analyzed, as more data was needed for the development of the model. This resulted in n=19-22 for these treatments. For the last three treatments n=4. Light intensity control group: 240 µmol m^{-2} s^{-1} . Light spectrum control group: R70:G10:B20. Treatments are named according to their deviation from the control group.

3.5.5. Dry weight

Lastly, the dry weight % was calculated during the pilot and the main experiment to analyze the effect of pre-harvest (5 days) treatments on Coriander, Mustard Frills Green, and Tatsoi Purple. This data was also used for the development of a PLS regression model that can predict dry weight % based on VIS-NIR spectrometry. As shown, the dry weight of individual species cannot be accurately predicted by the PLS regression model (Fig. H11). A better prediction can be made when a model was developed for the three species together. Such a model is however not useful for a grower.

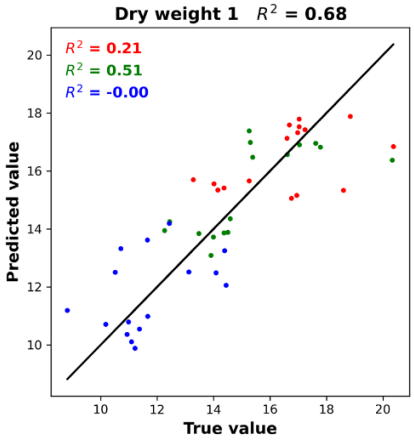


Fig H11. PLS regression model based on VIS-NIR spectrometry is not able to accurately predict the dry weight % (low-medium R^2). Dry weight % was measured by weighing the microgreens before and after they were dried in an oven for 24-48h at 105 °C. With this data the dry weight % was calculated ((dry weight/ fresh weight) *100%). Data of dry weight % as affected by pre-harvest (5 days) treatments (Control, 440 µmol m^{-2} s^{-1}, R90:B10, R60:B40, 140 µmol m^{-2} s^{-1}, 640 µmol m^{-2} s^{-1}, R50:B50) was used for the development of the model. Red represents Coriander, green represents Mustard Frills Green and blue represents Tatsoi Purple microgreens. For coriander between nine and thirteen replicates were used per treatment. For mustard and tatsoi per treatment eight replicas were used.

Appendix I – Literature review

Literature review

The vertical farming system with its ability to control the growing environment to a great extent could enable GROWx to produce products with the desired quality traits. For this, however, the proper growing conditions for the development of these traits must be known. This research will contribute to defining those. GROWx, Wageningen University (WUR), and the Amsterdam Institute for Advanced Metropolitan Solutions (AMS) are now developing a vertical farming system where the growth environment and energy costs are optimized, and the cultivation will be fully automized with the use of robotics and AI. This new and improved cultivation system will be called GROWx 2.0. To develop such a system, there is still a lot of research needed. In this research project, a study is conducted on the proper light conditions for the efficient production of high-quality products, to support the development of the GROWx 2.0 system. Before the start of this experimental study, information must be gathered on which quality attributes add value to the product. This, to determine the focus of the experimental study. Next, it must be known how these qualities could potentially be influenced by aspects of the light environment. Lastly, it is important to found out more about the biosynthetic pathways behind these effects, as this knowledge can be used to specifically target the synthesis of certain nutritional compounds. This information can then be used to design light treatments with the potential to improve the vertical farming system. To gather information on these topics a literature review was done.

The following research question was investigated: ''Which strategies have the potential to improve microgreen quality?'' To answer this question the following sub-questions come into mind:

- How can microgreen quality be defined?
- What compounds influence these quality attributes?
- How do the light environment influence these compounds and the quality attributes in microgreens, and how is growth affected?''

Consumer perception of microgreen quality

Quality is a combination of characteristics that give the product a high market value. The characteristics of a product that make up a valuable product, vary with personal preferences, and with the specific product. For fresh produce in general, characteristics that are important for quality are appearance (color, texture), taste, shelf life, and nutritional value (Nicole et al., 2019). Appearance is usually the initial attribute of a product that attracts consumers. Therefore, this attribute affects the initial choice of purchase. Other characteristics are, however, more important for consumer satisfaction and future purchase (Renna et al., 2017). For microgreens specifically, another study showed that consumer appreciation was mainly determined by flavor and texture, although visual appearance also played a role (Caracciolo et al., 2020). In addition, the shelf life of the product is important.

Taste is a complicated, but important quality attribute of microgreens. In research, it was found that flavor determines overall eating quality most in microgreens, compared to the texture and appearance (Xiao et al., 2015). These results are in line with the recent study of Tan et al. (2020). In this research project, it was found that overall liking of microgreens was strongly correlated to the taste of microgreens (r=0.83), which was a stronger correlation than was found for appearance (r=0.67) and smell (r=0.65) (Tan et al., 2020). This shows again that although texture, smell, and appearance all contribute to the perception of microgreens by the consumer, microgreen taste is the most important predictor.

Many different attributes make up the quality taste. It was found that lower astringency, sourness, and bitterness characteristics correlate with the higher consumer acceptability of microgreens (Caracciolo et al., 2020). It is assumed that a different correlation could be found when microgreens are tasted together with a complimentary dish. Recent research by Caracciolo et al. (2020) looked at the relationship between microgreen taste and the concentration of certain plant compounds. It was found that the phenolic content is tightly linked to astringency, sourness, and bitterness. This could be due to the bitter taste that is associated with many flavonoids, like naringin, isoflavone glucosides (Caracciolo et al., 2020). Another recent study found similar results. They showed once more that the phenolic content of microgreens is strongly correlated with flavor and eating quality. Next to the correlation between taste, eating quality, and phenols, a similar correlation between pH and these characteristics was found. The pH and total phenolic content values could therefore be used as indicators of sensory value and could be used to predict consumer acceptability (Xiao et al., 2015). Lastly, the sugar content was found to be an important contributor to microgreen taste because it affects sweetness, bitterness, and sourness attributes (Tan et al., 2020).

Next to sensory attributes, the nutritional value of microgreens is important for consumers, as it influences the perception of microgreen quality. It was found that, as consumers' health awareness increases, the nutritional value of food becomes more important in the consumers' purchasing (Xiao et al., 2015). It is therefore expected that the value of this quality attribute will increase in the coming years. Microgreens are highly nutritious greens, because of their high concentrations of nutrients and high antioxidant capacity, which may have health-promoting benefits related to the development of a vast array of inflammatory-related chronic diseases, and are therefore often called functional foods (Choe et al., 2018). As microgreens are so healthy, this aspect of quality perception could be used to increase sales. The study of Caracciolo et al. (2020) supports this argument. It was stated that the communication of the various health benefits of microgreens may contribute significantly to the market success of microgreens (Caracciolo et al., 2020).

Another question comes to mind: *'' What compounds influence these quality attributes? ''*

The compounds that influence these quality attributes

As mentioned, microgreen appearance, shelf life, taste, and nutritional value are important quality attributes. All are related to the compounds in the plant and their concentrations. Most knowledge about these compounds is related to the attribute nutritional value. It is known that the antioxidant capacity of compounds is an important contributor to this attribute and that it is reflected by the concentration of bioactive compounds in the plant, which include phenols, carotenoids, chlorophyll, and vitamins (Zhang et al., 2020). Some of these compounds also correlate with other quality attributes like appearance, shelf life, and taste. It is therefore interesting to look into these compounds.

Phenols, also called phenolics, are a group of compounds with an aromatic benzene ring with one or more hydroxyl groups. Plants mostly produce these compounds for protection against stress (Bhattacharya et al., 2010). Phenols are known to be potent radical scavengers, and as such have a high antioxidating effect. The consumption of phenols is known to have many health benefits, such as risk reduction of oxidative-stress-induced chronic diseases, such as cancer, cardiovascular disease, and type two diabetes. Besides this, phenols are known to be good antimicrobial agents (Alrifai et al., 2019). As mentioned, the amount of phenols in microgreens correlates with microgreen flavor. Phenols are, therefore, not only important for the nutritional quality of microgreens, but also the attribute taste (Caracciolo et al., 2020). Phenols can be divided into various groups.

An important group of phenols is the flavonoids, which can be further divided into subgroups including anthocyanins, flavonols, and flavones (Alrifai et al., 2019). Flavonoids are secondary metabolites that act as protective agents against UV radiation. In addition, they play a role in pollinating, pigmentation, and chemical defense against diseases (Alrifai et al., 2019). Anthocyanins, a subgroup of flavonoids create red, purple, and blue colors in plants, and thus influence the appearance of the plants. The compounds are mainly important for photoprotection in plants and have the potential to have strong anti-inflammatory effects (Alrifai et al., 2019).

Carotenoids are light-harvesting pigments located in chloroplasts. The compounds are thought to play an important role in maintaining health, and the prevention of several human diseases, among others cancer, eye disease, and diabetes (Zhang et al., 2020). The compounds collect light and pass the energy collected to chlorophylls. Carotenoid compounds play an important role in photosynthesis, have a photoprotective role, and are scavengers of reactive oxygen species, and thus have a high antioxidant capacity. Lutein, zeaxanthin, violaxanthin, and α- and β-carotene are some important carotenoid compounds. Carotenoids, like β-carotene and lutein, are synthesized via the isoprenoid pathway and are mostly found in leafy plant tissue (Alrifai et al., 2019). Another healthy compound, also located in chloroplasts, is chlorophyll. The reason for this is again, their anti-inflammatory effects, and antioxidant activity (Zhang et al., 2020).

Regarding nutrition, vitamins play an essential role in human nutrition and health and are very important to be included in the diet, as they cannot be synthesized by humans. Several vitamins have strong antioxidant potential, which makes them important for plants as well as humans (Asensi-Fabado & Munné-Bosch, 2010). Vitamin C and vitamin E are two, well-known and important vitamins. Earlier research showed a correlation between initial vitamin C level and shelf life in microgreens (Mir et al., 2017).

There are also plant compounds that harm human health. One of these compounds is nitrate. It is well known that high dietary nitrate consumption is associated with an increased risk of gastrointestinal cancer (Zhang et al., 2020). It is therefore beneficial to limit the amount of nitrate that accumulates in greens, if possible. The amount of nitrate that is largely present in microgreens is dependent on the species. In Swiss chard low accumulation of nitrates was found (< 1000 mg kg−1 fw), while moderate levels of nitrate were found in purple basil and radish (1000–2600 mg kg−1 fw), and high levels were found in coriander, cress, green basil, komatsuna, mibuna, pak choi and tatsoi (2600–4000 mg kg−1 fw). The risk associated with nitrate consumption is therefore different per species (Caracciolo et al., 2020).

What is interesting is that the amount of these compounds in plants can be influenced by environmental conditions. Plants respond to environmental stressors by activating a series of mechanisms. These mechanisms are similar to responses to pathogenic stimuli, which affect plant metabolism via the synthesis of phytochemicals, like the healthy compounds mentioned above. It seems therefore that specific environmental conditions could increase the synthesis of phytochemicals and enhance the nutritional value of microgreens. Interestingly, this effect of environmental stimuli on the synthesis of bioactive compounds is highest in young plants, as they have to adapt quickly to changing environmental factors to ensure survival. Microgreens are young plants and are therefore able to adapt quickly to changing environmental factors by enhancing the synthesis of phytochemicals. Furthermore, as microgreens in general already have a high nutritional value, the use of specific environmental conditions to increase the concentration of phytochemicals could be useful to improve their use as functional foods, which they are often called (Michell et al., 2020). It must be noted however that physical factors influence plant metabolism in a complex way, as often the expression of many genes is involved, which might make this a complicated task (Lobiuc et al., 2017).

An important environmental stressor that can affect the synthesis of bioactive compounds, is light. It seems that light intensity, light spectrum, and photoperiod, all can have an effect on the concentration of bioactive compounds in a plant (Alrifai et al., 2019). What is also interesting is that the light exposure history also affects a certain light treatment. For illustration: when buckwheat sprouts were exposed to LED blue light before they were exposed to UV-C light more bioactive compounds accumulated than when the plants were first exposed to UV-C light and were then exposed to blue light (Zhang et al., 2020). As in vertical farming, each aspect of the light environment can be controlled, it is interesting to look for ways to improve microgreen quality with specific LED light treatments.

A new question arises: *'' How do the light environment influence these compounds and the quality attributes in microgreens and how is growth affected?''*

Influence of LED light on yield and quality of microgreens, and the processes behind these effects

Plants can be influenced by many aspects of the light environment. The most important being light intensity, light spectrum, and photoperiod. The intensity of the light that the plant receives influences the growth and quality of a plant. In general, increasing light intensity increases the photosynthetic rate, and thus the growth of plants. It is however also widely known that high light intensity can cause damage to a plant. The optimal light intensity for a plant is species-specific. Furthermore, it is known that different wavelengths of light can have particular effects on plants. In the following section, a brief overview is given of the main colors of light that are used in horticulture and their effects.

Red light (600 – 700 nm) light matches the absorption maxima of chlorophylls and is therefore very important for photosynthesis. Furthermore, red light is absorbed by phytochrome receptors which have an active and an inactive form, as is far-red light (700 – 750 nm). Red light is absorbed by the inactive form of phytochrome and transforms the compound into an active form. Far-red light does the exact opposite and is absorbed by the active form of phytochrome and transforms the compound to the inactive form. The ratio between red and far-red light, therefore, determines the ratio between active and inactive phytochrome. Phytochrome is related to germination, stem elongation, leaf expansion, flowering induction, and more. Red light is associated with biomass increase, higher phenolic contents, and activation of the antioxidant system. (Lobiuc et al., 2017). When plants are solely subjected to red light, ''red light syndrome'' might be caused. Red light syndrome has a negative effect on the plant. It lowers the photosynthetic capacity and thus limits growth. Red light also has a positive effect on plants. It was for instance found that red light has a positive effect on the vitamin C content of microgreens (Zhang et al., 2020). Red light also increases the concentration of phenols in plants compared to white light, however not as much as blue light does (Litvin et al., 2020). In turn, far-red light is known to increase photochemical efficiency. This effect is larger when far-red light is supplied in combination with light with shorter wavelengths.

Green light (500 – 600) was once ignored as green light is not absorbed as much by chlorophylls. However, it has now been shown that green light penetrates the leaf further than blue and red light. Therefore, green light increases carbon fixation and possibly yield, even though it is not absorbed as much by the plant. On the other hand, it was also found that green light could reverse blue and UV-B mediated stomatal opening, which can have a negative effect on photosynthesis (Alrifai et al., 2019). At this point the exact effects of green light in horticulture systems are unknown. A recent study concluded however that high-intensity green LEDs in combination with red and blue LEDs increased growth parameters in brassica microgreens (Kamal et al., 2020).

Blue light (400 – 500 nm) is sensed by cryptochromes and phototrophins, which regulate de-etiolation, phototropism, chloroplast movement, endogenous rhythms, growth of roots, light-induced stomatal opening, redox balance, and more. In addition, blue light induces stomata opening, which improves CO2 fixation, and thus increases photosynthesis. Furthermore, in a research project, it was observed that under blue light the cotyledon development was promoted, which could also be a result of the blue light-induced stomatal opening, or could be related to higher content of nitrogen in the leaves, as blue light is known to enhance the nitrate reductase activity (Lobiuc et al., 2017). Blue light also plays a role in anthocyanin, carotenoid, and chlorophyll production, and can be used to prevent hypocotyl elongation (Zhang et al., 2020). It was found that broccoli microgreens are grown under blue light, R0:B100, produced higher and more nutrient-dense microgreens (Kamal et al., 2020). Although blue light can also increase phenolic synthesis, it does not do so linearly with the increase of blue light (Litvin et al., 2020).

Blue and red light complement each other. When used together they yield better results than when each of these colors was used monochromatically for cultivation (Lobiuc et al., 2017). To illustrate this, it was found that a higher net photosynthetic rate was found when the ratio between red and blue light decreased. This is likely due to the increase of photosynthetic parameters, and the elevation of chloroplast development when blue light is added to red light (Zhang et al., 2020). To maximize the positive effects of the light environment on microgreens it is however important to find the most suitable ratio of red to blue light, which is species-specific (Lobiuc et al., 2017).

UV-light (100 – 400 nm) is electromagnetic radiation just outside of the visible spectrum. UV radiation can be classified into three different categories: UV-C (100 – 280 nm), UV-B (280 – 315 nm), and UV-A (315 – 395 nm) radiation. UV-C radiation is harmful to organisms due to the tissue permeability of this light and the ability to modify DNA. UV-B radiation can also be harmful. This type of UV light can cause damage to DNA, membranes, and proteins (Alrifai et al., 2019). Moderate dosages of UV light might be beneficial, however, as they induce the synthesis of flavonoids, for photoprotective activity (Zhang et al., 2020). UV-A is the least damaging UV light (Alrifai et al., 2019).

Lastly, photoperiod also affects plant development and growth. Photoperiod and light intensity effects on plants can be similar, as they both cause a change in the number of photons that are subjected to light in a certain period. It was found that this change in the number of photons is sometimes the actual cause of the effect of photoperiod (Alrifai et al., 2019). Again, optimal photoperiod is species-dependent.

Next, the most effect on light on the most important compounds in plants regarding quality as defined earlier will be touched upon. The effect of light on yield will also be mentioned as in commercial production systems, yield is very important. An analysis of the current knowledge on the way this is presented below.

Phenolic compounds

In several microgreen species, an increase in the production of phenols occurred at high light intensities. In tatsoi and kohlrabi peak production of total phenols was found at a light intensity of 440 and 545 µmol m^{-2} s^{-1}. In red pak choi, the highest accumulation of phenols was found between 440-330 µmol m^{-2} s^{-1} (Samuoliene et al., 2013). Other research had similar findings and stated that low light intensity downregulated the phenols produced. This effect was not present for all species, as it was shown that increasing light intensity decreased the flavonoid content and total phenolic content (TPC) in Orthosiphon simaneus, which is a herb (Alrifai et al., 2019). Also, in the research of Samuoliene et al. (2013) for some species, no increase of phenols was found at higher light intensities. Mustard, for

instance, showed no difference in total phenols for high light intensities up to 545 µmol m^{-2} s^{-1}, compared to the normal light intensity of 220 µmol m^{-2} s^{-1} (Samuoliene et al., 2013).

Another study researched the effect of a very high light intensity treatment for one day on sprout quality. This treatment was called a. It was found that when 700 µmol m^{-2} s^{-1} was subjected to plants for 24h, after which plants were allowed to recover for one day before they were harvested, the phenolic content increased. In alfalfa sprouts double the amount of ferulic acid was found, which was significant. Furthermore, an increase of 83% was found for sinapic acid in broccoli sprouts. Lastly, myricetin accumulated under the high light treatment. The reason for this was either that the total phenolic content increased or the decrease of phenolic content during aging slowed down. Unfortunately, a decrease in fresh weight seemed to be related to the light shock treatment, slightly decreasing the usefulness of this treatment in commercial cultivation systems. No difference in dry weight was observed (Oh & Rajashekar, 2009).

Light spectrum also affects the phenols produced in the plant. In lettuce, it was found that blue light enhanced TPC and antioxidant activity (Alrifai et al., 2019). In other research similar results were found for buckwheat and Chinese cabbage. The study concluded that this effect could be due to the shorter wavelengths of blue light, that have higher photon energy. The higher photon energy of these wavelengths induces higher levels of photooxidative stress, which results in a higher accumulation of phenols, as these compounds protect the plant from photo-induced damage (Zhang et al., 2020). Another research dived deeper into the biochemical pathways that resulted in this effect. It was found that blue light plays an important role in the production of phenols. The phenylalanine ammonia-lyase (PAL) enzyme is important in the photoinduction of the synthesis of phenols. It was found that blue light plays a role in the regulation of this enzyme. Blue light namely regulates the inhibition of PAL by the transformation of hydrocinnamic acids from the trans-form to the cis-form, which is less inhibitory with the result that more phenols are being produced (Lobiuc et al., 2017). Besides this, a study found that sprouts grown under blue and white LED light had higher expression levels of PAL and F3'H compared to sprouts grown under red LED light. It was also found that several gene expression levels were upregulated (C4H, CHI, FLSII, ANS). In Chinese cabbage especially blue light was effective in enhancing the expression of F3'h, compared to white and red LED light (Alrifai et al., 2019)

Red light can also increase the concentration of phenols in microgreens. When microgreens grown with HPS light were subjected to red light of 210 µmol m^{-2} s^{-1} + 90 µmol m^{-2} s^{-1} HPS lightning three days before harvest the phenols concentration increased in 9 of the 10 microgreen species, compared to plants grown under 300 µmol m-2 s-1 HPS lightning. Increases ranged from 9.1% in mustard to 40.8% in tatsoi. In amaranth, a decrease of 14.8% was found (Samuoliene et al., 2012). In general, however, it was found that a combination of red and blue light is more effective to increase the synthesis of phenolic compounds than monoaromatic red or blue light. This is likely caused by the fact that chlorophyll a and b absorb both red and blue wavelengths, which increases the synthesis of compounds in general, and by the fact that red and blue light together can modulate different pathways for different syntheses (Alrifai et al., 2019). The responses of plants to red and blue light regarding phenolic content are however dependent on species and environmental conditions. The study on the effect of light on two basil species, acyanic (green) basil and cyanic (red) basil, showed that the phenolic synthesis in red basil was most stimulated under higher ratios of blue light, while green basil was most stimulated under higher ratios of red light. It is thought that this difference is the result of different regulatory mechanisms of the PAL enzyme, which is important for the synthesis of phenols, in green and red tissues. It was proposed that this could be due to the involvement of phytochrome in green plant tissues, and a possible co-regulation of PAL and anthocyanin accumulation

under the effect of light. Furthermore, there could be a difference in the regulation of gene expression of light between red and green tissues (Lobiuc et al., 2017).

Flavonoids

It was found that blue light increases the flavonoid content of Chinese kale and buckwheat sprouts. Red light had the opposite effect (Zhang et al., 2020). In a research project, a significant correlation between flavonoid content and gene expression of PAL, 4CL, and CHS enzymes were found. Therefore, this effect of LED light could be due to differences in gene expression. It seems that LED lights may modulate genes that encode these enzymes (Alrifai et al., 2019). In the study, UV-B light also led to an increased flavonoid content. In broccoli sprouts even an increase of 92% was found, compared to sprouts grown under white LED light (Zhang et al., 2020). Another study showed similar results. It was found that UV light in open field conditions resulted in larger amounts of flavonol and anthocyanin compounds (Alrifai et al., 2019). Furthermore, UV-B light systematically increased the isoflavone, a flavonoid compound in soybean sprouts, while it was decreased in red clover sprouts. It is thought that nitric oxide signaling induces isoflavone biosynthesis by increasing the gene expression of the key enzymes involved, which include PAL and CHS. It was found that the influences of UV-B are dependent on the wavelength, light intensity, and species (Zhang et al., 2020).

Flavones and flavonols are classes of phenolic compounds. Both are regulated by blue and UV light through chalcone synthase, which is the key enzyme in this pathway and induces CHS and mRNA accumulation in exposed tissues. Several phenolic acids were upregulated by UV light. UV light is perceived by the same receptors as blue light. It is therefore likely that the effect of blue light with wavelengths close to UV light has a similar effect as UV light. Furthermore, the higher amount of these phenolic compounds under blue light might be caused by the cytochrome P45 which leads to reactive oxygen species (ROS) accumulation. As a result, phenolic acids are produced as a protective mechanism. Likely, this is also the reason that high phenolic content is associated with high antioxidant activity (Lobiuc et al., 2017).

Anthocyanins

Pigments are compounds that can absorb and reflect light energy, anthocyanin is such a compound. Anthocyanins play an important role in photoprotection. Therefore, they protect the plant against damaging light (Lobiuc et al., 2017). When present in high amounts, the pigment makes tissues reddish or purple. Research showed that anthocyanin production is related to light intensity. In pak choi and tatsoi, anthocyanin production increased 1.3 and 1.5 times respectively under 330 µmol m^{-2} s^{-1} compared to 220 µmol m^{-2} s^{-1}. In kohlrabi anthocyanin levels led to the highest anthocyanin content. In mustard, anthocyanin production was lower under 220 µmol m^{-2} s^{-1} compared to lower and higher light levels, which shows a species-specific response (Samuoliene et al., 2013). This is supported by another research project that found that the anthocyanin content of red amaranth microgreens increased at 280 µmol m^{-2} s^{-1} compared to 230 µmol m^{-2} s^{-1}, while the anthocyanin content of leafy vegetable amaranth was not affected by the light intensity in the range of 130 – 280 µmol m^{-2} s^{-1} (Meas et al., 2020). This could potentially be due to different regulatory pathways, as described by Lobiuc et al. (2017). Furthermore, in red leaf lettuce, a study found that the anthocyanin content doubled when the plants were exposed to a light intensity of 440 µmol m^{-2} s^{-1} for three days before harvest, compared to 220 µmol m^{-2} s^{-1}(Samuoliene et al., 2013). It seems therefore that this could be a good strategy to increase anthocyanin content in plants. However, as mentioned effects are thought to be species-specific.

Blue light can also increase anthocyanin biosynthesis. In lettuce, it was found that Delphinidin-3-glucoside, which is an anthocyanine, was highest in the lettuce grown under light with a relatively high blue content (RB 53:47 and RB 58:42 compared to RB 89:11). Levels of two other anthocyanins, cyanidin-3- and peonidin-3-glucoside, remained unchanged. Another study in lettuce, also showed that blue light increased the anthocyanin content. It was found that blue light upregulated the phenylpropanoid enzyme, which had a positive effect on the biosynthesis of anthocyanin. As a result of the increase in anthocyanin in lettuce, the leaves became reddish (Alrifai et al., 2019). Also, pre-harvest light seems to work to increase the anthocyanin content. In red leaf lettuce, the anthocyanin content doubled when plants were exposed to light with a high blue light content (69%) for three days before harvest (Gómez & Jiménez, 2020). UV-A light has similar effects regarding the accumulation of anthocyanin. In lettuce, an increase of 11% was found in the anthocyanin content, when supplemental UV-A light was used. UV-A light and blue light are both dependent on cryptochromes and have similar wavelengths. Therefore, they have similar effects on the plants when used during cultivation (Alrifai et al., 2019). Green light has been shown to decrease the blue light accumulation of anthocyanins, while far-red light and blue light enhanced the accumulation of anthocyanins with increasing intensity in kale, broccoli, and beet microgreens. Furthermore, a study showed that green light could reverse cryptochrome activation by blue light. It is thought that this is done by the conversion flavosemiquinone signaling states, which were initiated by CRY1 and blue light, to inactive states by green light. This theory was confirmed by an experiment where plants were grown under blue light, or blue light + green light, which resulted in a decreased anthocyanin content (Alrifai et al., 2019).

Red light can also affect. It was found that when microgreens grown with 300 µmol m^{-2} s^{-1} HPS light were subjected to red LED light with an intensity of 210 µmol m^{-2} s^{-1} + 90 µmol m^{-2} s^{-1} HPS lightning three days before harvest the anthocyanin concentration increased in broccoli (45.1%), kale (44.0%), amaranth (38.0%), tatsoi (34.5%), parsley (27.0%) and pea (14.6%). In borage (51.8%), mustard (45.1%), and beet (43.3%) a decrease in the total amount of anthocyanins was found. In basil anthocyanin concentration was not affected (Samuoliene et al., 2012). Looking at the research of Meas et al. it seems that both red and blue light are important for the synthesis of anthocyanins. In this research, the highest anthocyanin content was found for the red + blue spectra (R70:B30) followed by white, red and blue LED light respectively, for red amaranth. In leafy vegetable amaranth, it was found that both red + blue (R70:B30) and white LED light resulted in significantly higher anthocyanin content, compared to monochromatic blue and red light spectra (Meas et al., 2020). The idea that anthocyanin content is related to both red and blue light is supported by the research of Lobiuc et al. (2017), in which it was stated that anthocyanin synthesis is mediated by both red and blue light receptors (Lobiuc et al., 2017). It could be that blue light similarly affects anthocyanin as other phenols, and that red light affects anthocyanin content via a different pathway.

Lastly, photoperiod also affects anthocyanin content. In red amaranth and leafy vegetable amaranth, anthocyanin content increased with increasing photoperiod. For red amaranth, the maximum anthocyanin content was found at a photoperiod of 16h, while for leafy vegetable amaranth the optimum was 20h (Meas et al., 2020).

Carotenoids

Increased light intensity seems to affect the carotenoid content of microgreens. Research has shown that increased light intensity could increase the concentration of carotenoids in microgreens (Alrifai et al., 2019; Choe et al., 2018). In another study, however, no significant effect was observed for two amaranth microgreen species tested with light intensities in the range of 130 – 280 µmol m^{-2} s^{-1} (Meas

et al., 2020). It could be that carotenoid content is only influenced at a higher light intensity, or that the effect of light intensity on carotenoid content is species-specific.

Light spectrum also affects the carotenoid content of microgreens. It was found that blue light could increase the amount of carotenoid present. In mustard, beet, and parsley microgreens a higher carotenoids content was found when the plants were subjected to 33% blue light, compared to microgreens grown in the dark, and with lower blue light percentages. Not all carotenoids were increased with higher percentages of blue light however. Furthermore, it was found that UV light can also stimulate the production of carotenoids, which is likely caused by the fact that blue and UV light works via the same photoreceptors (Alrifai et al., 2019). In addition, yellow light (595 nm) can increase the carotenoid content in microgreens. In tatsoi microgreens, an increase of 16% was found when supplemental yellow light was used (Zhang et al., 2020). Lastly, in mustard microgreens, it was found that supplementary green LED light, in combination with red and blue light, could enhance β-carotene and the lutein/ zeaxanthin ratio (Alrifai et al., 2019). Other research projects found similar results (Berba & Uchanski, 2012; Choe et al., 2018). Light spectrum does not always affect carotenoid content however. In a study with two amaranth microgreen species light spectrum did not have a large effect on the carotenoid content. In red amaranth, no difference in carotenoid content was found between microgreens grown under white, red, blue, or red + blue LED light. In leafy vegetable amaranth only, a significant difference was found between microgreens grown under red light and white LED light, in which higher carotenoid content was found for microgreens grown under red LED light. This carotenoid content was however not significantly different from blue LED light (Meas et al., 2020).

Photoperiod also affects the amount of carotenoid in microgreens. In a study with two amaranth microgreen species, it was found that carotenoid content increased with increasing photoperiod (Meas et al., 2020). Another research found an increase of 50% for antheraxanthin, and 133% for zeaxanthin for mustard microgreens that were exposed to 463 µmol m-2 s-1 for 36h. The plants also showed a significant reduction in the amount of chlorophyll which indicates light stress, which is likely the reason for the increase of carotenoid compounds as they act as photo protectors (Kopsell et al., 2012).

Chlorophyll

Chlorophyll is a pigment that absorbs mainly red and blue regions of light, 663 nm – 642 nm and 430 nm – 453 nm respectively. The pigment is a very important compound for photosynthesis and is therefore related to the concentration of sugars that are produced in a plant (Lobiuc et al., 2017). In turn, the amount of sugar in the plant is related to the taste of microgreens. Chlorophyll might, therefore, be an indicator for sugar production, and possibly taste. Moreover, the appearance of microgreens is positively influenced by the amount of chlorophyll in the leaves. In a research project, it was found that microgreens with a higher chlorophyll content had a more vibrant color, further enhancing the importance of chlorophyll for quality (Tan et al., 2020).

High-intensity light can decrease the chlorophyll content of microgreens. In mustard microgreens, a decrease of 27% was found when microgreens were cultivated with 463 µmol m^{-2} s^{-1}, in comparison to 275 µmol m^{-2} s^{-1} (Zhang et al., 2020). Contradicting this, are the results of Meas et al. (2020), who found that the leaf chlorophyll index increased at higher irradiance levels, 545-440 µmol m^{-2} s^{-1}, compared to normal conditions, 220 µmol m^{-2} s^{-1}, in red pak choi and mustard. Another study investigating the effect of light intensity on amaranth microgreens found no effect of light intensity on chlorophyll content. They, however, only measured chlorophyll content with light intensities up to 280 µmol m^{-2} s^{-1} (Meas et al., 2020). Chlorophyll content could therefore be more affected at higher light intensities. More research must be done to determine the exact effects of light intensity on chlorophyll content.

Regarding the light spectrum, it was found that blue light drives chlorophyll production, as blue light improves the gene expression of certain genes that are involved in chlorophyll production, such as FeCH, GluTR, and MgCH. It is also thought that blue light regulates some enzymes in the chlorophyll synthesis pathway. Some of these enzymes are ALA-dehydratase, aminolevulinic acid (ALA)-synthase, phosphoenolpyruvate (PEP)-kinase, dioxovalerate (DOVA)- dehydrogenase, and DOVA-transaminase. Likely, as a result of this, it was found that a light spectrum of R6:B1 increased the chlorophyll level in Chinese cabbage. Red light on the other hand leads to a reduction of 5-aminolevulinic acid which is a tetrapyrrole precursor that is required for chlorophyll synthesis. In addition, in Chinese cabbage, it was found that red light reduced the amount of ALA, Proto IX, Mg-Proto IX, and protochlorophyllide, which are chlorophyll precursors (Lobiuc et al., 2017). In practice, it seems that a combination of blue and red light is important for plant health and chlorophyll content. For broccoli microgreens, it was found that a light spectrum of R80:B20 could be used to increase total chlorophyll content (Zhang et al., 2020). For amaranth microgreens, a study found that a light spectrum of R70:B30 increased the amount of chlorophyll compared to white, blue, or red LED light. The lowest amount of chlorophyll was found for the microgreens grown under red LED light (Meas et al., 2020). To efficiently increase the chlorophyll in microgreens it is therefore important to research the optimal ratio between red and blue light in each species.

Photoperiod also affects the chlorophyll content in microgreens. This effect is again species-specific. In a research project with two amaranth microgreen species, it was found that for red amaranth a photoperiod of 16h resulted in the highest level of chlorophyll. For leafy vegetable amaranth microgreens, the highest chlorophyll content was found at a photoperiod of 20h, which was the highest photoperiod in this experiment. The maximum chlorophyll b content was obtained at 16h for both microgreen species (Meas et al., 2020).

Vitamins

As mentioned two important vitamins for human health are vitamin C and vitamin E, as they have strong antioxidant potential (Asensi-Fabado & Munné-Bosch, 2010). In this literature study only these two vitamins will be talked upon.

Ascorbic acid (vitamin C) concentrations did not seem to be affected that much by light intensity (Samuoliene et al., 2013). In a study on the effect of light intensity on two amaranth microgreen species, no effect of light intensity on ascorbic acid content was found. In the same project also no effect of photoperiod was found (Meas et al., 2020). In the research of Samuliene et al. (2013) the ascorbic acid content of two of the species tested was actually influenced by light intensity. In red pak choi and tatsoi, however, the ascorbic acid content was 3.8 and 3.5 times as high at 110 µmol m^{-2} s^{-1} compared to 220 µmol m^{-2} s^{-1} (Samuoliene et al., 2013).

Light spectrum does seem to affect ascorbic acid content. It was found that red light can increase the amount of ascorbic acid in microgreens. When microgreens grown with HPS light were subjected to red LED light of 210 µmol m^{-2} s^{-1} + 90 µmol m^{-2} s^{-1} HPS lightning three days before harvest the amount of ascorbic acid increased in some microgreen species, compared to plants grown under 300 µmol m^{-2} s^{-1} HPS lightning. In amaranth ascorbic acid content increased 79.5%, in pea it increased 65.2%, in kale 60.6%, in broccoli 59.1%, and in mustard it increased 25%. In tatsoi, beet, and parsley microgreens no significant effect was found (Samuoliene et al., 2012). Again, species react differently to the different light spectra they were subjected to. Also, species that are very closely related, seem to respond differently to the different light spectra. In red amaranth, a blue light spectrum resulted in a significantly higher ascorbic acid content compared to a white, red + blue, and a red spectrum. The lowest ascorbic acid content was found with a red light spectrum. For leafy vegetable amaranth, only

a significant difference was found for the red light spectrum in which a lower ascorbic acid content was found, compared to the other spectra. The ascorbic acid synthesis of this microgreen species did not react to blue light, while the ascorbic acid synthesis of red amaranth did react (Meas et al., 2020).

Lastly, photoperiod also has an effect on the vitamin C content. When plants were subjected to continuous light before harvest, vitamin C content was affected in lettuce, rocket, and spinach. In wild rocket, preharvest continuous light (5 days) resulted in a large increase of vitamin C content. Ascorbic acid content was 2.8 times higher for treatment with continuous white LED light, and 3,5 times higher for the continuous blue LED light treatment (Nicole et al., 2019).

Vitamin E, α tocopherol, is also affected by light. A light intensity of 110 µmol m^{-2} s^{-1} resulted in a higher α tocopherol content, compared to 220 µmol m^{-2} s^{-1} in red pak choi, tatsoi, kohlrabi, and mustard. In mustard, however, also high light intensities, 545 and 440 µmol m^{-2} s^{-1} resulted in higher α tocopherol accumulation compared to 220 µmol m^{-2} s^{-1} and in kohlrabi, the highest concentration of α tocopherol was found under 440 µmol m^{-2} s^{-1}. Interestingly, at 545 µmol m^{-2} s^{-1} negligibly low concentrations of α tocopherol were found in kohlrabi (Samuoliene et al., 2013). Therefore, although vitamin E seems to be effect by light intensity, no clear relationship between light intensity and α tocopherol accumulation seems to be present.

Antioxidants

In some studies, the total antioxidant capacity of the microgreens was measured to determine their nutritional value. The antioxidant capacity is determined by the abundance of compounds that have antioxidant potentials such as phenols, carotenoids, chlorophyll, and vitamins. As the antioxidant capacity as affected by light treatments provides additional information on potential strategies to improve the quality of microgreens, the information found in the literature on this subject is provided below.

In borage microgreens, it was found that under 440 µmol m^{-2} s^{-1} the antioxidant protective system was stimulated, and the highest amount of antioxidants phytochemicals was found. Higher light intensity, 545 µmol m^{-2} s^{-1} suppressed the antioxidant levels in the microgreens (Viršilė & Sirtautas, 2013). In the study of Meas et al. (2020) antioxidant capacity increased when light intensity increased. In this project, however, only light intensities up to 280 µmol m^{-2} s^{-1} were measured.

Light spectrum also affected the antioxidant capacity in microgreens. In amaranth microgreens, the highest antioxidant capacity was observed for microgreens grown under a red + blue spectrum, compared to a white, blue and red LED spectrum. The lowest antioxidant capacity was found for microgreens grown with the red LED spectrum (Meas et al., 2020). Pre-harvest light spectra could also increase the antioxidant capacity. In a study that researched the effect of several pre-harvest LED treatments in red-leaf lettuce, it was found that plants that were exposed to LED light with high blue light content (69%) for four days before harvest, had an increased antioxidant capacity. These had a 61% and 47% higher antioxidant capacity, compared to the plants that were exposed to the ultraviolet light treatment and plants of the control group, respectively (Gómez & Jiménez, 2020).

Lastly, a research project found that increasing the photoperiod increased the antioxidant capacity in amaranth microgreens. The highest antioxidant capacity was found at a photoperiod of 16h (Meas et al., 2020).

Sugar content

In plants, sugars are important for growth, and light signaling pathways. In some plants, high sugar content in leaves is associated with a reduction in nitrates. Sometimes, however, high sugar concentrations are also associated with a decrease in photosynthetic rate (Samuoliene et al., 2013).

Sucrose concentration is also related to LED radiation. In kohlrabi, tatsoi, and red pak choi the lowest sugar content was found under the lowest radiation (220 µmol m^{-2} s^{-1} and 110 µmol m^{-2} s^{-1}). The highest sugar content for kohlrabi was found at 545 µmol m^{-2} s^{-1} (11,4 times higher as compared to normal light conditions 220 µmol m^{-2} s^{-1}), in red pak choi at 440 µmol m^{-2} s^{-1} (6 times higher) and in tatsoi at 330 µmol m^{-2} s^{-1} (9,5 times higher). It seems therefore that higher levels of radiation result in higher sugar content. However, no linear relationship between the two is found for all species (Samuoliene et al., 2013).

Nitrate

It was found that nitrate concentration is related to light intensity. A negative correlation was found between the nitrate content and the light intensity. The lowest light intensity resulted in the highest nitrate concentrations (Samuoliene et al., 2013). In a similar study with lettuce, it was found that a higher light intensity leads to a reduction of nitrate accumulation in lettuce leaves (Gómez & Jiménez, 2020). Another research project found that moderate light levels of 330 – 440 µmol m^{-2} s^{-1} resulted in the lowest nitrate levels in borage microgreens (Viršilė & Sirtautas, 2013).

Furthermore, green LED light was able to decrease the amount of nitrate in radish microgreens. Again, however, this is species dependent. It was found that the amount of nitrate in basil microgreens was increased with green light, which is of course not beneficial (Zhang et al., 2020). Red light also seems to be effective to decrease the amount of nitrate in microgreens. Research demonstrated that when Shiso microgreens were subjected to pre-harvest (3 days) supplemental red lightning that the nitrate content of the plant decreased. In addition, the antioxidant level of the plant increased, due to the increase of ascorbic acid and anthocyanin concentration. (Brazaitytė et al., 2013). Another study on the effects of pre-harvest light also found that red-white spectrum affects the amount of nitrate in lettuce, rocket, and spinach. In wild rocket a large decrease of nitrate was observed for red-white continues pre-harvest light (Nicole et al., 2019). In contrast, it was found that blue light could increase the nitrate content in plants (Gómez & Jiménez, 2020).

Shelf life

Another important quality attribute of microgreens is their shelf life. Unfortunately, not that much is known about the influence of LED light on the shelf life of microgreens. The research of Nicole et al. (2019) did take this attribute into account when researching the effect of LED light on microgreen quality and found that when microgreens were exposed to light with a high blue content, 35%, a few days before harvest, the shelf life of baby spinach and rocket was increased by several days (Nicole et al., 2019). As these greens generally have a short shelf life, an increase like this can already be quite meaningful.

Taste

Not much research has been done on the effect of light on taste. It is a difficult attribute, as it is known that secondary metabolites, essential oils, and phenolic compounds all influence the aromas and flavors in culinary plants. It seems though that LED light can affect the taste, as it is known that some of the compounds that play a role in microgreen taste are influenced by the light environment. It was for instance found that the accumulation of secondary metabolites, essential oils, and phenolic compounds increased under blue or ultraviolet light (Litvin et al., 2020). Some research projects did

directly show the effect of light on taste. It was found that light spectrum, as well as light intensity, affect microgreen taste (Litvin et al., 2020; Nicole et al., 2019). As taste is influenced by many different compounds, it is difficult to predict the effect of LED light on the perceived taste of microgreens.

Yield

Yield is a very important parameter, as revenue is tightly associated with it. Yield is determined by many different factors, among them are various light influences. Plants have photoreceptors with which they harvest light energy to drive photosynthesis or respond to light quality and quantity changes (Alrifai et al., 2019).

It was found that higher light intensity can have a beneficial effect on the yield of microgreens. In red pak choi and tatsoi, a higher dry weight percentage was found for 545 and 440 µmol m^{-2} s^{-1} compared to 220 µmol m^{-2} s^{-1}. It was however also found that the hypocotyl length of mustard, tatsoi, and kohlrabi was lower at these light intensities, compared to the normal light intensity of 220 µmol m^{-2} s^{-1}. In another study, similar results were found. It was found that fresh weight and leaf area increased, and hypocotyl length decreased with increasing light intensity, from 110 to 440 µmol m^{-2} s^{-1} in borage microgreens. In this research, a light intensity of 545 µmol m^{-2} s^{-1} resulted in inhibited biomass growth (Viršilė & Sirtautas, 2013). The research of Samuoliene et al. (2013) also researched the effects of light intensity on plant yield. In the study, it was concluded that the optimal radiation for brassica microgreens lies between 320 – 440 µmol m^{-2} s^{-1}. Here also nutritional value was taken into account (Samuoliene et al., 2013).

Light spectrum also affects microgreen growth. Red and blue light matches the absorption of chlorophyll a and b, and are therefore most important for photosynthesis, and thus growth (Alrifai et al., 2019). Monochromatic red light is known to elongate hypocotyls and cotyledons in lettuce, which is a phytochrome-dependent process. This effect can be adjusted with additional blue light (Alrifai et al., 2019). Ying et al. (2020) found that for plant height a negative correlation between the percentage of blue light in the spectrum and the size of microgreens, in kale and mustard. When the blue light percentage was increased from 5 until 30%, a linear reduction of plant size was found in the microgreens that were studied. The fresh weight did not decrease. Moreover, it was found that cotyledons were darker red in red-leafed mustard under higher blue light percentages. In cabbage, green, and arugula, which are green-leafed species, the plant showed less pure-green cotyledon when subjected to higher blue light percentages, affecting the appearance of these microgreens. In cabbage microgreens, blue light percentages did affect fresh weight. An increase in yield at 15% blue light was found. At higher and lower blue light percentages lower yield was found. This, in combination with acceptable cotyledon color and the fact that no effect on the hypocotyl length and leaf area was found for 15% blue light, led this study to conclude that for cabbage the optimal light spectrum is R85:B15. For arugula, kale, and mustard they recommended R95:B5, as here the highest hypocotyl length and largest leaves were found in combination with no negative effect on fresh or dry yield (Ying et al., 2020). It must be noted however that blue light can also have a positive effect on microgreen yield, as blue light is related to higher stomatal opening and thus higher photosynthesis (Lobiuc et al., 2017). Another research project that investigated the effect of light spectrum on growth, found that when plants were exposed to far-red light (25% of the spectrum), microgreens obtained a larger yield compared to plants cultivated with red-white light, or blue light. Far-red light exposure also resulted in a higher percentage of dry weight (10% versus 9%) (Nicole et al., 2019).

As already shown by the research of Ying et al. (2020) the optimal light spectrum is dependent on the microgreen species cultured. In the recent research project of Kamal et al. (2020), which was dedicated to finding the optimal light spectrum for brassica microgreens, it was found that brassica microgreen

varieties grown under LEDs lightning with a spectrum of R70:G10:B20 enhanced to hypocotyl length, leaf area, fresh weight, and dry weight % compared to R80:B20, R20:B80 and R20:G10:B70. For nutritional content a spectrum of R80:B20 was optimal. The researchers concluded that a spectrum of R70:G10:B70 was the optimal spectrum for brassica microgreens when nutritional value, growth, and yield all were taking into account (Kamal et al., 2020). In amaranth, a study that compared the effect of monochromatic white, red, blue light, and an R70:B30 spectrum found that the yield of red amaranth and vegetable amaranth increased under the spectrum with red and blue light. For leafy vegetable amaranth, however, the fresh yield was the same for microgreens grown with red or red plus blue light (Meas et al., 2020). In coriander, a study observed the highest biomass when plants were cultivated with a light spectrum of R10:B1 (Lobiuc et al., 2017).

Recently it has been found that changing the light environment a few days before harvest (also called preharvest light) can be successful in improving microgreen growth and quality. A study was conducted to research the effect of several pre-harvest LED treatments in red-leaf lettuce. Plants were grown under red and blue LEDs (R87:B13) for 24 days. Next, the plants were treated with one of the following LED treatments for 4 days: ultraviolet-A light, high blue light (69%), or high intensity (440 µmol m-2 s-1) radiation. In this experiment, no difference in fresh weight was observed between the treatments and the control treatment for one of the varieties that was used. The other showed a reduced fresh weight for the blue light treatment. This was likely due to the fact that the plants were subjected to the treatment when they were still in the exponential growth phase. It is thought that when plants are subjected to this treatment after this phase, this effect will not be presented. In this variety, it was also found that the high light intensity treatment could increase fresh weight. These plants received more than double the DLI and as a result produced 50g more than the plants that were treated with the ultraviolet treatment (Gómez & Jiménez, 2020).

Lastly, also photoperiod affects microgreen yield. It was found that a higher photoperiod resulted in a higher photosynthetic capacity compared to a shorter photoperiod in lettuce and watercress. There was, however, no clear link between light duration and accumulation of nutritional compounds. The effect of photoperiod is likely caused by the increase in the quantity of photons that are emitted within a period. Daily light integral, which describes the number of photosynthetically active photons that are delivered to the plants within 24h, is known to be linearly correlated with crop yield and the synthesis of phytochemicals (Alrifai et al., 2019).

A study investigated the effect of 14 µmol m-2 s-1 blue LED light overnight in mustard and arugula microgreens during the whole growth period. It was found that this treatment resulted in stem elongation by 16% for mustard and 10% for arugula, and crop yield for 32% and 29% respectively. In addition, cotyledon area and leaf mass per area, and cotyledon color were enhanced in both species. No effect on total chlorophyll, carotenoid, and phenolic contents was found (Ying et al. 2020).

Then, what does this all say about the potential strategies to increase microgreen quality?

Conclusion and potential strategies to optimize microgreen yield and quality

In conclusion, the light environment can be used to steer microgreen growth and quality. It is however clear that the optimal light environment is different for each microgreen species, and affects quality attributes differently. In literature, a lot of information on the effect of specific light treatments on compounds in microgreens is present. It becomes clear that for the optimization of each attribute, another light environment is optimal. Blue light can for instance increase the concentration of many nutritional compounds, including phenols, which also affect taste (Alrifai et al., 2019; Caracciolo et al.,

2020). On the other hand, blue light is known to potentially decrease yield and increase the nitrate content, which has a negative effect on the quality of microgreens (Gómez & Jiménez, 2020; Ying et al., 2020). Striving for the optimal light treatment is therefore always a balancing act of different strategies. To improve the overall quality of microgreens it seems that a combination of blue and red light is very important, in combination with the light intensity and photoperiod. The optimal balance of these light attributes is however species-dependent. In conventional cultivation systems, with many different species, it would be very difficult to optimize the growth and quality of microgreens efficiently because of it. In the GROWx 2.0 system, this is could be less difficult because of the potential of data-driven farming and robotics. Still, it would be quite a challenge, as at this point the optimal light environment for each species cultivated in the GROWx system is not known. Therefore, extensive research must be done to determine the effects of LED light on each of the species cultivated.

For GROWx, especially the use of preharvest light seems to have a lot of potential for increasing microgreen quality while remaining a high yield, as research has shown that pre-harvest light treatments can have a positive effect on microgreens. Furthermore, pre-harvest light treatments are easy to implement. Mainly, pre-harvest light treatments with high light intensity and high blue content seem to work (Kamal et al., 2020; Lobiuc et al., 2017; Samuoliene et al., 2013; Zhang et al., 2020). It is important to note that this is mostly based on nutritional content, as most literature focuses on this quality attribute. There is though, also evidence that microgreen taste and shelf life are also affected by light treatments (Litvin et al., 2020; Nicole et al., 2019). As results are very species-specific, and depend on the combination with other environmental factors, an experiment is proposed where these pre-harvest light treatments are tested in the GROWx system. It is already clear that after this research project, more research on the light environment must be done to optimize the light environment at GROWx. Therefore, this experimental study should be combined with the development of a spectrometry model that can predict the abundance of important plant compounds that are related to quality. This model could in the future be used to speed up experimental studies on the optimal light environment, as it would provide a quick and cheap method to get insight into microgreen quality.