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FACULTY OF SCIENCE AND TECHNOLOGY

BACHELOR THESIS

Study programme / specialisation:

Biological chemistry

The spring semester, 2022

Open

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Thesis title: The effect of different light sources on physical attributes and biochemical compounds in Norwegian cucumbers as a function of storage

Credits (ECTS): 20

Keywords: Cucumbers LED HPS Quality Storage Pages: 58

Stavanger, 31.05.22

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Abstract

The cucumber is an important crop for greenhouse cultivation in northern latitudes such as in Norway, where light and temperature requirements are not met year around for cucumber cultivation. Producing a consistently satisfactory crop and influencing favorable consumer traits including physical attributes and biochemical compounds is therefore of key concern. Light quantity and quality are principal factors determining the development and subsequent quality of crops such as the cucumber and emerging research indicates that light emitting diodes can improve both levels of biochemical compounds and physical traits in crops as opposed to traditional high pressure sodium lamps in a greenhouse environment. The manipulation of these factors has potential for prolonging desired traits both in terms of taste and nutritional enhancement and physical parameters in cucumbers across storage and presents an intriguing concept. Examining how light treatments combining overhead light emitting diodes and highpressure sodium lamps with intracanopy light emitting diodes affect two different parthenocarpic smooth cucumber cultivars, IMEA and DeeRect with regards to a selection of physical and chemical parameters when freshly harvested and after 4 weeks of storage was therefore the focus of this thesis. A taste test was also conducted to possibly correlate the experimental findings with consumer taste preference. The physical parameters measured consisted of length, diameter, weight and perceived color measurements, whilst the chemical parameters consisted of dry matter content, soluble solid content, pH, total titratable acid content measurements, vitamin C content, pigment content and ionic content.

Both the physical and chemical parameters expressed variability to different light combinations in both cultivars, though the IMEA cultivar was affected to a greater extent. The use of light emitting diodes had a positive effect on both physical and chemical parameters particularly in the IMEA cultivar, though both cultivars had similar declining results in response to storage and only perceived color for the IMEA cultivar and the carotenoid content in the DeeRect cultivar appeared to exhibit different values after storage as a function of light treatment. The DeeRect cultivar expressed higher overall favorable values for the measured parameters indicating the importance of cultivar choice on set parameters.

ii

Acknowledgements

I would like to express my appreciation to NIBIO Særheim for giving me the opportunity to work with this thesis and the employees there who have both facilitated and supported my work with it. I would like to thank my supervisors at NIBIO Særheim, Michel Verheul and in particular Dmitry Kechasov. His constructive insights, thorough guidance and engagement, both in the laboratory and in terms of literary suggestions and review made the completion of this thesis possible. In addition, I would also like to thank Niroz Juma, and Jack Eide for their assistance with the experimental aspects at NIBIO Særheim. I would also like to thank my supervisor at UiS Cathrine Lillo for her valuable remarks and assistance.

Finally I would like to thank my family and partner for their support during a busy and challenging time.

Abbreviations

| SL | Supplemental lighting |
|-----------------|---|
| HPS | High pressure sodium lamps |
| LEDs | Light emitting diodes |
| NIBIO | Norwegian Institute for Bioeconomy Research |
| PAR | Photosynthetic active radiation |
| PPFD | Photosynthetic photon flux density |
| SSC | Soluble solid content |
| TTA | Total titratable acids |
| DMC | Dry matter content |
| IC | lon chromatography |
| HPLC | High pressure liquid chromatography |
| IC-CD | Ion chromatography coupled with conductivity detector |
| ppm | Parts per million |
| RH | Relative humidity |
| acid meq.factor | Acid milliequivalent factor |
| MAE | Malic acid equivalents |
| DCP | 2,6-Dichloroindophenol |
| ANOVA | Analysis of variance |
| NR | Nitrate reductase |

Index

| 1. Introduction | 1 |
|--|----|
| 1.1 Background of the thesis | 1 |
| 1.2 Physiology and biology of the cucumber | 2 |
| 1.3 Effect and quality of light | 3 |
| 1.4 Quality parameters of cucumbers | 5 |
| 1.4.1 Storage as a parameter of cucumber quality | 7 |
| 1.5 Pigments | 8 |
| 1.6 Ionic content | |
| 1.6.1 Ion Chromatography | 9 |
| 2. Objectives | 11 |
| 3. Materials and Methods | 12 |
| 3.1 Design | |
| 3.2 Growth conditions | 14 |
| 3.2.1 Atmospheric conditions and nutrient supply | 14 |
| 3.2.2 Light | 15 |
| 3.3 Chemicals and equipment | 18 |
| 3.4 Physical measurements | 20 |
| 3.5 Taste test | 20 |
| 3.6 Chemical measurements | 21 |
| 3.6.1 DMC, TTA and SSC | 21 |
| 3.6.2 Vitamin C | 23 |
| 3.6.3 Pigments | |
| 3.6.4 Ionic content | 27 |
| 3.7 Statistical methods | 29 |

| 4. Results | <u>3</u> 0 |
|--------------------------------------|------------|
| 4.1 Physical measurements | |
| 4.1.1 Length | |
| 4.1.2 Diameter | |
| 4.1.3 Weight | |
| 4.1.4 Color | 35 |
| 4.2 Chemical measurements | |
| 4.2.1 DMC | <u>3</u> 9 |
| 4.2.2 SSC | 39 |
| 4.2.3 pH, TTA and TTA/SSC | 40 |
| 4.2.4 Pigments | |
| 4.2.5 Vitamin C | 42 |
| 4.2.6 Ionic content | |
| 4.3 Taste test | 48 |
| 5. Discussion | |
| 5.1 Results of physical measurements | |
| 5.2 Results of chemical measurements | |
| 6. Conclusion | |
| 7. References | <u>5</u> 5 |
| 7.1 Literature | |
| 7.2 Web pages | |

1. Introduction

1.1 Background of the thesis

The cucumber *Cucumis sativus* is the third most cultivated food crop (Du *et al.*, 2022) with an annual production of approximately 91.26 million metric tons worldwide in 2020 (Statista, 2019). Greenhouse cultivation of *C. sativus* utilizing supplemental lighting (SL) is of particular importance in northern Europe (Fujiwara *et al.*, 2016), providing both an extended harvesting season and optimized growing conditions from control of atmospheric and climatic conditions. Light quality and quantity are known to be key factors influencing both yield and crop quality (Fujiwara *et al.*, 2016; Hasan *et al.*, 2017) and optimizing these parameters would have both great economic and to a further extent environmental significance.

The possibility of exchanging traditional high pressure sodium lamps (HPS) with more energy effective light emitting diodes (LEDs) is becoming a feasible alternative due to the decreasing prices of LEDs (Verheul *et. al.*, 2021). The use of LED SL has previously largely been confined to intracanopy lighting in conjunction with overhead HPS, due to the significant capital investment of using LED overhead lighting. Mitchell (2015) has pointed out that using LEDs as overhead SL in a greenhouse environment fails to capture some of the advantages of LEDs, as the LED fixtures would have to be placed high above the canopy to minimize the fixtures blocking large amounts of incoming solar radiation. Overhead LEDs would subsequently have to be placed in dense arrays high above the canopy to provide enough radiation constituting an unnecessarily costly operation. The contribution of natural sunlight in Norway during wintertime however is almost neglectable (Verheul *et. al.*, 2021), presenting an economical opportunity for overhead LED lighting during the winter months. In addition, this lack of sunlight provides an ideal location to examine the effects of solely supplemental light on greenhouse crops such as cucumber. It is therefore important to research the possible

physiological and chemical responses in cucumbers cultivated under different light treatments such as LED overhead lighting as opposed to HPS overhead lighting to discover possible differences in quality parameters such as appearance and flavor guality, which play paramount roles in customer preference and therefore commercial value. In addition, examining the nutrient content of *C. sativus* cultivated under different combinations of HPS and LEDs may be proven fruitful for optimizing these levels. Evaluating potential differences in shelf life of cucumbers grown under different light regimes presents the main novelty of this thesis, adding to the potential economic and environmental advantages of cucumber cultivation by light manipulation. The research was conducted at The Norwegian Institute for Bioeconomy Research (NIBIO) facility Særheim located in Jæren approximately 26 kilometers south of the City of Stavanger. This is a major agricultural area in Norway owing to its relatively flat topography and mild oceanic climate. Jaeren is also a key area for greenhouse cultivation in Norway and is NIBIOs primary area for greenhouse research at their facility Saerheim. This research is part of an initiative by the Norwegian Agricultural Association to make cucumber cultivation in Norway more sustainable and competitive and is funded by The Research council of Norway and by "The GRO-fund" established by the BamaGroup AS.

1.2 Physiology and biology of the cucumber

C. sativus is a subtropical climbing plant originating from northern India and has been cultivated for over 3000 years (Haifa Group, 2021). It is therefore accustomed to and prefers growing in the ideal temperature range of above 20-24°C and is not frost tolerant (Agri Farming, 2022). Due to the extensive breeding of *C. sativus*, a multitude of varieties have been bred from which the fruit differ in both appearance and physiology (Figure 1). *C. sativus* cultivars can be both monoecious and gynoecious in which monoecious cultivars produce both staminate "male" and pistillate "female" flowers on the same plant and gynoecious cultivars produce predominantly pistillate flowers. As

fruits are only produced on pistillate flowers, gynoecious cultivars have become more predominant in cultivation as they are more productive. Both monoecious and gynoecious varieties of *C. sativus* require pollination to produce fruits and contain seeds. This challenge has led to the creation of parthenocarpic *C. sativus* hybrids that can produce fruits without pollination and are seedless. These hybrids are not pollinated and as a result are long and smooth. Greenhouse varieties popular in Europe are parthenocarpic and gynoecious resulting in highly productive plants (Haifa Group, 2022).



Figure 1. Different varieties of *C. sativus* fruit depicting both non-parthenocarpic spiny cucumbers (left) and parthenocarpic smooth fruits (right) (Wikipedia Commons, 2009; Wikipedia Commons 2014).

1.3 Effect and quality of light

Light is a key factor for plant growth affecting both the rate of photosynthesis and plant productivity (Blom and Ingratta, 1984; Fan *et. al.*, 2008; Zoratti *et al.*, 2014). Plants utilize light in the 400-700 nm range of the electromagnetic spectrum referred to as photosynthetic active radiation (PAR). To measure PAR, photosynthetic photon flux density is used (PPFD) which measures micromoles of photons per square meter (µmol $m^{-2} s^{-1}$) (Short and Coles, 2001). Even though natural daylight may be sufficient during the spring and summer months at northern latitudes, introducing supplemental lighting either overhead or to the inner canopy has been shown to add the advantages of improving fruit quality, extending the growing season, or enabling year-round production and importantly sustaining a stable workforce (Heuvelink *et al.*, 2006). HPS overhead lamps have been used extensively as SL for greenhouse cultivation due to their relative low cost compared to other light sources and adequate intensity and spectrum for plant growth (Kozai *et. al.*, 2016). In northern climates the significant proportion of heat created from HPS lamps can be advantageous during the colder months as subsequent heating can be reduced. Brault *et al.* (1989) estimated that as much as 41% of the heat requirements for greenhouse cultivation could be provided from HPS lamps in northern climates. There are several downsides with HPS lamps however including high life-cycle costs, intense environmental impact (Nelson, 2012) and the thermal radiation they emit, rendering them unsuitable for intracanopy lighting.

The use of LEDs as SL offers new and intriguing agricultural possibilities as they emit considerably less infrared radiation than conventional HPS lamps (Särkkä *et al.*, 2017). This enables LEDs to be used as intracanopy lighting without burning the leaves, thereby increasing PAR around the plant. Intracanopy SL can even be utilized in areas that receive sufficient sunlight year around to increase yields and produce a more uniform crop year around (Kozai *et. al.*, 2016). In addition, LEDs can be manufactured to emit a narrower and more focused light spectrum than HPS lamps, increasing PAR per joule of electricity consumed. The most efficient colors with regards to PAR per joule electricity for LEDs are blue, red and cool white (Nelson and Bugbee, 2014), thus many LEDs used as supplemental lighting in greenhouses have been manufactured solely with diodes emitting these colors as seen in Figure 2.



Figure 2. *C. sativus* cultivated with overhead HPS lamps and intracanopy LEDs (left) and with overhead LEDs and intracanopy LEDs (right) at NIBIO Saerheim.

Emerging research indicates that blue and red spectral LEDs also have the ability to increase the accumulation of both primary and secondary metabolites compared to white light (Hasan *et. al.*, 2017). It is also discovered that red or blue LEDs have the potential to delay senescence of fruits by a reduction the production of ethylene and ascorbates (Ma *et al.*, 2014). The possibility of increasing nutrients, beneficial secondary metabolites such as antioxidants and modulating metabolism to increase shelf-life coupled with the increased efficacy of LEDs make them an attractive and viable option to both supplemental intracanopy lighting and for overhead lighting to increase fruit quality in a greenhouse environment.

1.4 Quality parameters of cucumbers

The main parameters of cucumber quality can be divided into commercial quality, nutrient quality and flavor quality (Zhang, J. *et. al.*, 2021). Commercial quality is primarily characterized by visual appearance as it is the first parameter of quality made by the consumer. Principal factors influencing quality perception are uniform shape and size (Mitcham *et. al.*, 1996). It should be mentioned that other parameters affecting commercial quality vary across different geographical areas such as presence of spines on the fruit which are preferred in Asia, whilst spineless fruits are preferred in Europe (Chen *et al.*, 2014). Color is also an important parameter of quality for the consumer and is determined by the levels of chlorophylls in the exocarp (skin).

Cucumbers consist primarily of water at approximately 96% by weight with the primary macronutrients constituting sugars at approximately 1.7 % by weight (OFG, 2022). The nutrient content of cucumbers can be determined by their soluble solid content (SSC) which include sugars, organic acids, amino acids phenolic compounds, soluble pectins and minerals. Since the main soluble solids in fruits are sugars, SSC can be used as an estimate for sugar content (Mitcham et. al., 1996). Cucumbers contain relatively few micronutrients, mainly vitamins A and C as well as some calcium and iron (Szalay and McKelvie, 2022). The antioxidants in fruit and vegetables, which includes vitamin C, can reduce oxidative stress and are known to offer nutritional benefits for consumers (Kozai et. al., 2016). Light quality can affect the photo-oxidative properties of plants by modulating antioxidant defense systems resulting in increased antioxidative enzyme activity (Hasan et al., 2017). The effect of light quality on the production of vitamin C is increasing as Verkerke et. al., (2014) found that irradiating only tomato fruits with blue and red LEDs increased vitamin C content logarithmically with increasing radiation exposure when compared to a control group. Comparable results in cucumbers would be notable and increase incentive to use LEDs in cucumber cultivation. Sweetness and sourness are two major taste attributes affecting the palatability of cucumbers (Du et. al., 2022) and are attributed to the sugar and acid content of the fruits respectively. As previously mentioned, SSC is an acceptable indicator of total sugar content in fruits and the amount of total titratable acids (TTA) is a good parameter of acidity or sourness in fruits. The proportion of TTA to SSC may indicate consumer preference, as Johnsen (2012) demonstrated that consumer preference of cucumbers may be negatively correlated to higher values of TTA over SSC.

1.4.1 Storage as a parameter of cucumber quality

Storage conditions are known to play a major role in the preservation of cucumber quality (Verheul *et. al.*, 2013). Cucumber fruits are as previously mentioned largely composed of water and have sufficient number of stomata on the exocarp of mature fruits to necessitate wrapping in plastic to prevent excess transpiration of water prior to sale (Sui *et. al.*, 2017). Cucumbers are also prone to yellowing at storage temperatures exceeding 15 °C, but are prone to chilling injury below 10 °C (Dhall *et. al.*, 2011). Careful control of storage temperature between these temperatures is therefore an important factor to prolong cucumber quality.

It has also been discovered that an increase of chlorophyll content in the exocarp seems to be correlated with increased shelf life in cucumbers (Kowalczyk *et al.*, 2018), making chlorophyll content a significant parameter of improved storage and commercial interest. Dry matter content (DMC) is another parameter of possible commercial value as it could have a positive impact on fruit keeping quality according to Särkkä *et. al.*, (2017). Verheul *et. al.*, (2013) found that SSC levels decline in cucumbers, but TTA increases as a result of storage, possibly reducing consumer preference. Examining the potential effect of different light sources on how levels of SSC and TTA alter over time in cucumbers might therefore provide useful results for maintaining consumer preference.

1.5 Pigments

Terrestrial plants including *C. sativus* mainly use the specific chlorophylls *a* and *b* in addition to carotenoids to construct pigment-protein complexes. Chlorophyll *a* and *b* exhibit separate roles in photosynthesis where chlorophyll *a* is the principal light capturing pigment, absorbing light and driving oxygenic photosynthesis, whilst chlorophyll *b* is an accessory pigment that absorbs light and transfers the corresponding energy to chlorophyll *a*. The absorption maxima of chlorophyll *a* and *b* respectively are 429 nm and 659 nm for chlorophyll *a*, and 455 nm and 642 nm for chlorophyll *b* (Kume *et. al.*, 2018; Campbell *et. al.*, 2017; Panawala, 2017) shown in Figure 3. Carotenoids also function as accessory pigments broadening the light spectrum for photosynthesis with three typical absorption maxima in the 400-500 nm range (Lichtenthaler and Buschmann, 2001) depicted in Figure 3. They also act as photoprotective agents, absorbing and dissipating excessive light, a crucial ability as excessive light would damage chlorophylls and create reactive oxygen species that are detrimental to cells (Campbell *et. al.*, 2017).



Wavelength of light (nm)

Figure 3. Absorbance spectrum depicting relative absorbance of chlorophyll *a*, *b* and carotenoids across different wavelengths of the electromagnetic spectrum. Chlorophyll *a* has two absorption maxima at 429 nm and 659 nm, chlorophyll *b* has two absorption maxima at 455 nm and 642 nm and carotenoids have three absorption maxima in the range 400-500 nm (Graf Commons, 2019).

1.6 Ionic content

The ions of inorganic salts play multiple roles in plant physiology and biochemistry, from regulating membrane permeability and osmotic pressure to being an integral part of the cellular membrane and are involved in different metabolic processes (Talbott and Zeiger, 1998; Hirsch and Sussman, 1999). The main inorganic ions in most plants can be divided into the cations: K^+ , Ca^{2+} , Mg^{2+} , Na^+ and NH_4^+ , whilst the main anions constitute: Cl^- , NO_3^- , HPO_4^{2-} and SO_4^{2-} (Cataldi *et. al.*, 2003). The inorganic ionic content of cucumbers in response to different light quality and treatments is not well understood and further research may provide insights towards the effect of light on mineral absorption and allocation in *C. sativus* plants and fruits and further indicate altered biochemical processes.

1.6.1 Ion Chromatography

The principals of chromatography rest on the interactions of a stationary phase and mobile phase. The analytical mixture is carried by the flow of a mobile phase through the stationary phase, where the separation of the different components in the analytical mixture rely on the differences in migration rates among the mobile phase components. Ion chromatography (IC) is a type of high-pressure liquid chromatography (HPLC) in which an eluent carries the analytical mixture constituting the mobile phase. Separation is achieved in a column which holds the stationary phase and consists of ion exchange resins that retain different ionic species in the mobile phase to varying degrees. Coupling IC to a conductivity detector (IC-CD) creates a reliable analytical method for ionic determinations (Cataldi *et. al.*, 2003). Both qualitative and quantitative information regarding ionic content in cucumbers can be obtained by IC-CD at very minute concentrations of parts per million (ppm) in a single run. Qualitative information regarding ion species is acquired by separate retention times of peaks in the chromatogram acquired from standard solutions with a known ionic profile. Quantitative

information regarding the corresponding concentration of each ion is given by the integral of separate ion peaks in the chromatogram derived from the standard solutions with known ionic concentration and is calculated by computer software (Skoog *et. al.*, 2017). To reduce ionic background noise from the eluent and obtain higher sensitivity of measurement the use of a post-columnar suppressor prior to conductivity detection is used (Weiss, 1995).



Figure 4. Ion chromatography setup for analysis of cations with eluent (top flask), conductivity detector (green box on shelf) and column heater containing column (right black box) at NIBIO Saerheim.

2. Objectives

The main objective of this thesis is to examine potential differences overhead LEDs may have compared to overhead HPS both with and without intracanopy SL on a selection of physiological and chemical parameters of cucumber fruits, and to which extent storage affects these parameters as a response to different light treatments. Two cultivars of parthenocarpic smooth cucumbers, namely IMEA and DeeRect, will be subjected to the same light treatments to examine the attribution of only cucumber cultivar on the results and how different cultivars may respond to the same treatments. In addition, a taste test will be performed on one cultivar to discover potential differences in preference due solely to light treatment.

The physiological parameters that will be assessed aim to reflect the most apparent quality parameters for consumer preference and will consist of length, diameter, weight and perceived color measurements. The chemical parameters assessed will also aim to include important consumer preference such as SSC, pH and TTA, but also factors that may reflect improved storage qualities such as DMC and chlorophyll concentration. Since inorganic ionic concentration may give insights into biochemical and physiological processes such as photosynthetic rate potentially affecting the chemical parameters DMC and SSC and to some extent physical parameters in addition to the extent of water and nutrient allocation, the content and respective concentrations of the most predominant inorganic anions and cations in the cucumber fruits will be examined by IC-CD.

3. Materials and methods

3.1 Design

The cucumber cultivars IMEA and DeeRect were cultivated in the same greenhouse compartment. Both cultivars were grown under overhead HPS lamps and separated by a light blocking blind from the same cultivars grown under overhead LED lights. Half of the cucumbers grown under HPS and LED overhead lighting had additional intracanopy LED lighting.

The experimental design can be divided into physical measurements, chemical measurements and a taste test. The physical measurements were first conducted on a set of six freshly harvested cucumbers from each light treatment and cultivar, harvested between 08.02.22 – 10.02.22, and were replicated after approximately 4 weeks in a dark environment with an average 90% (± 10%) relative humidity (RH) and 12 °C. Three different fruit replicates from each light treatment and cultivar were used for the chemical measurements. They were frozen at -80 °C when freshly harvested between 09.02.22 – 11.02.22 and the same six fruit replicates used for the physical measurements were frozen at -80 °C after 4 weeks to preserve the chemical constituents and cease metabolism in the fruits. DMC, pH and TTA measurements were conducted in one step on thawed and homogenized cucumbers, which were further centrifuged to create a supernatant for SSC and IC-CD measurements. The homogenized cucumbers and supernatant from each parallel of each treatment were stored at -20 °C for further analysis of chlorophyll content. Vitamin C measurements were conducted on supernatant from thawed and homogenized freshly harvested cucumbers from each light regiment and cultivar, subsequently only three fruit replicates for vitamin C were measured. The taste test was also only conducted with freshly harvested cucumbers, but from only the IMEA cultivar with three separate fruit

replicates from the physical and chemical measurements for each light treatment. Figure 5 is a flow diagram representing the above-mentioned design.



Figure 5. Flow diagram representing the experimental design. The different colored boxes represent the different segments of the design in which orange represents the taste test, blue represents the physical measurements and green represents the chemical measurements.

3.2 Growth conditions

3.2.1 Atmospheric conditions and nutrient supply

The cucumbers grown under the different light treatments were exposed to the same atmospheric conditions as they were grown in the same greenhouse compartment and the light blocking blind did not transect the whole compartment, allowing for free airflow. The cucumbers used in the experiments were harvested in the period 08.02.22-11.02.22 and the atmospheric conditions consisting of and incoming solar radiation from 25.01.22-11.02.22 are listed in Table 1 to account for conditions applicable during the timeframe of fruit development.

Table 1. Average atmospheric conditions and incoming solar radiation (W m⁻²) for the greenhouse compartment housing the *C. sativus* plants used in the experiments. The measurements were taken in successive intervals of 5 min over the timeframe 25.01.22-11.02.22.

| Relative humidity (%) | Temperature (°C) | CO ₂ concentration (ppm) | Solar radiation (W m ⁻²) |
|-----------------------|------------------|-------------------------------------|--------------------------------------|
| 73.33 | 22.47 | 1039.64 | 23.12 |

The *C. sativus* plants were grown in rock wool medium with a nutrient solution prepared from the fertilizers listed in Table 2.

Table 2. Fertilizers used in the nutrient solution given to the cucumbers diluted into 500 L of water including the volume of each fertilizer, with the manufacturers name and corresponding weight and nutrient composition.

| Name | Weight (kg) | Nutrient composition (% w/w) |
|------------------------|-------------|--|
| YaraTera Calcinit | 50.0 | 15.5 N; 15.8 Ca |
| Pioner Rød (NPK | 50.0 | 8.7 N; 4.5 P; 29.9 K; 3.5 Mg; 4.6 S |
| makro) | | |
| YaraTera Krista MgS | 7.5 | 9.6 Mg; 13 S |
| Pioner Mikro PL.M/Jern | 11.2 | 0.32 B; 0.13 Cu; 1.62 Fe; 0.63 Mn; 0.06 Mo; 0.32 |
| | | Zn |
| Haifa MPK | 2.0 | 22.7 P; 28.7 K |

3.2.2 Light

Three different light sources were used in the experiment; two different overhead ones and one intracanopy source as listed in Table 1.

Table 3. Light sources used in the experiment with names, supplier, light source with placement.

Respective efficacy in $\mu mol \ W^{\text{-1}}$ listed by the suppliers is also included.

| Name | Supplier | Light source | Placement | Efficacy (µmol W ⁻¹) |
|--------------------------------------|------------|--------------|-------------|----------------------------------|
| MASTER GreenPower 600W, 400V, E40 | Philips | HPS | Overhead | 1.67 |
| SolarMass 300, M27 | Solar Mass | LED | Overhead | 2.7 |
| GreenPower LED, Interlighting 3.0 | Philips | LED | Intracanopy | 3.0 |

The corresponding light spectrums of the overhead light sources the intracanopy LED light source were measured, and the resulting light spectrum graphs are depicted in Figure 6 and 7.



Figure 5. Light spectrum graph depicting absolute irradiance (μ W cm⁻² nm⁻¹) of both overhead the HPS light source and the overhead LED light source across the wavelengths (nm) approximately 375-890. The overhead HPS light source is represented by the blue function with a narrow and tall peak in the at 825 nm infrared region. The HPS light source also shows significant irradiance between 550-750 nm. The overhead LED light source is represented by the brown function with a broad peak at approximately 675 nm in the visible region and has a higher proportion of irradiance in the 450-550 nm region than the HPS overhead light source, with another peak at 450 nm (J. Eide, personal communication, 5 May, 2022).



Figure 6. Light spectrum graph depicting absolute irradiance (µW cm⁻² nm⁻¹) of the LED intracanopy light source. This light source consisted of only blue and red spectrals and as a result irradiance is confined to a relatively small, but broad peak at 450 nm and a larger and broad peak at 675 nm. The flat peak at 675 nm is due to overexposure (J. Eide, personal communications, May 5, 2022).

PAR quantification for the corresponding light sources and listed in Table 2.

Table 4. PAR quantification measurements in PPFD (µmol m⁻² s⁻¹) for overhead HPS, overhead LED and intracanopy light sources used. Measurements at various positions of two plants from both IMEA and DeeRect cultivars for the overhead light sources and only in the middle for the intracanopy light source are included. The top position refers to the top of the plants, the middle refers to 90 cm above the rock wool growing medium and bottom refers to the top of the rock wool medium (J. Eide, personal communication, 6 April, 2022).

| Light source | Position | PPFD (µmol m ⁻² s ⁻¹) |
|-----------------|----------|--|
| Overhead HPS | Тор | 273.5 |
| | Middle | 47.5 |
| | Bottom | 34.5 |
| Overhead LED | Тор | 183.0 |
| | Middle | 35.5 |
| | Bottom | 20.0 |
| Overhead HPS + | Middle | 62.5 |
| Intracanopy LED | | |
| Overhead LED + | Middle | 58.0 |
| Intracanopy LED | | |

3.3 Chemicals and equipment

The chemicals and equipment used in the chemical measurements are listed in Table 5 and Table 6 respectively.

Table 5. All chemical used in the chemical measurements with corresponding names, CAS numbers, vendors and product numbers.

| Chemical | CAS number | Vendor | Product number |
|---|--------------|---------------------------|----------------|
| Citric acid | 77-92-9 | VWR Chemicals, USA | 84841290 |
| Oxalic acid dihydrate | 6153-56-6 | Merck KGaA, Germany | 1004950500 |
| Sodium bicarbonate | 144-55-8 | Sigma-Aldrich, USA | 6297 |
| 2,6-Dichloroindophenol, sodium salt hydrate | 1266615-56-8 | Thermo Scientific, USA | 152870250 |
| Ascorbic acid standard | 50-81-7 | Sigma-Aldrich, USA | MAK075E |
| Sodium carbonate | 497-19-8 | Sigma-Aldrich, Germany | 7795 |
| Acetone | 67-64-1 | Merck KGaA, Germany | 1000202500 |
| Nitric acid | 7697-37-2 | Sigma-Aldrich, Germany | 695041 |
| 2,6-Pyridinedicarboxylic acid | 499-83-2 | Sigma-Aldrich, USA | 63808 |
| Sulfuric acid | 7664-93-9 | VWR Chemicals, USA | 450061 |
| Multi Anion Standard 1 for IC | - | Sigma-Aldrich, Germany | 69734 |
| Multi Cation Standard | - | Spectrascan, Sweden | SS-2877S |

Table 6. All equipment used in the chemical measurements with description, corresponding name and vendor.

| Instrument | Name | Vendor |
|-----------------------|-----------------------|------------------------|
| Digital pipette | Multipipette E3x | Eppendorf, USA |
| Digital refractometer | Refractometer PR-101α | Atago, Japan |
| Centrifuge | Jouan B4i | Thermo Scientific, USA |

| Vortex mixer | Vortex-Genie 2 | Scientific Industries, USA |
|-----------------------------|--------------------------------|---------------------------------|
| Titrometer | 794 Basic Titrino | Metrohm, Switzerland |
| Magnetic stirrer | 728 Stirrer | Metrohm, Switzerland |
| Magnetic stirrer and heater | ARE Hot Plate Stirrer | VELP Scientifica, Italy |
| lon chromatograph | 883 Basic IC plus | Metrohm, Switzerland |
| Column heater | ESA CH-150 | Analytical Instruments LLC, USA |
| Autosampler | 863 Compact Autosampler | Metrohm, Switzerland |
| lon suppressor | MSM A | Metrohm, Switzerland |
| Anion separation column | Metrosep A Supp 5 – 150 x 4 mm | Metrohm, Switzerland |
| Anion guard column | Metrosep A Supp – 5 x 4 mm | Metrohm, Switzerland |
| Cation separation column | Metrosep C4 – 150 x 4 mm | Metrohm, Switzerland |
| Cation guard column | Metrosep C4 – 5 x 4 mm | Metrohm, Switzerland |
| Membrane filter | Nylon filter 0,45 µm | VWR, USA |
| Syringe filter | Nylon syringe filter 0,45 µm | VWR, USA |
| Spectrophotometer | Multiskan Go | Thermo Scientific, USA |
| Microwell plate | Microplate VIS, 96/F-PS | Eppendorf, USA |
| Water deionizer | ELGA PURELAB Option-R7 | ELGA Labwater, UK |
| Spectrophotometer software | SkanIT RE ver. 5 | Thermo Scientific, USA |
| lon chromatograph software | MagIC Net ver. 3.2 | Metrohm, Switzerland |
| Statistical software | SPSS ver. 26 | IBM, USA |

3.4. Physical measurements

The length of the six cucumbers replicates for each light treatment and cultivar was measured using a ruler and the diameter was measured using a gage. The cucumbers were subsequently weighed and compared to a color scale depicting different cucumber assigned increasing numbers from 5 - 9 in accordance with perceived greenness (Figure 8).



Figure 7. Color scale from 5 – 9 used for color comparison of cucumbers in terms of perceived greenness.

3.5 Taste test

The taste test was conducted two times with a weekly interval on the 24.02.22 and the 03.03.22 and was single blinded. For each light treatment of the IMEA cultivar three fruit replicates were sliced and mixed to minimize potential errors arising from the use of an unrepresentative cucumber. Two cucumber slices from the four light treatments were placed on plates marked into quarters with different numbers for each light treatment.

The test subjects were employees at NIBIO Særheim at a total of n = 23 for the two times. A questionnaire was handed to the test subjects asking them to score taste preference of each light treatment on a scale from (-3) - 3 in rising integers of 1 whole number, where -3 signified the least preference and 3 signified the most. A comment box was also included for each light treatment.

3.6 Chemical measurements

3.6.1 DMC, TTA and SSC

DMC, TTA and SSC determination was performed on thawed cucumbers that were homogenized using a handheld blender until a smooth and almost homogeneously colored fluid was obtained. DMC was determined gravimetrically by drying a sample of homogenate with known weight in an incubator at 105 °C until constant weight was achieved (approximately two days).

TTA can be determined experimentally by titration of a known volume of fruit juice with a 0,1 N NaOH solution to and endpoint of pH 8.2 using a potentiometric electrode (Mitcham *et. al.*, 1996). To calculate TTA, the predominant acid of the fruit needs to be determined which is malic acid in cucumbers, constituting 64% of the total organic acid content in sliced cucumber (Verheul *et. al.*, 2013). This acid is assumed to constitute total acid content and TTA. Malic acid is a diprotic acid with a molar mass of 134.09 g/mol. The normality of malic acid is therefore 67.05 N known as the equivalent factor. As the volume of NaOH solution added is given in mL, the equivalent factor is multiplied with a factor of 1000 to give the acid milliequivalent factor (acid meq.factor) as described in Equation 1. and is equal to 1 mN.

acid meq. factor(Malic acid) =
$$\frac{\left(\frac{134.09}{mol}\frac{g}{mol}\right)}{1000} = 0.067 \, mN$$
 Equation 1.

TTA is assumed to equal malic acid equivalents (MAE) by fresh weight which is described in Equation 2. The factor of 100 is a conversion factor to give % MAE by fresh weight.

$$\% MAE = \frac{mL(NaOH) \times N(NaOH) \times 0.067 \times 100}{mL(juice titrated)}$$
 Equation 2. (Mitcham *et. al.*, 1996)

% MAE = Malic acid equivalents in % per fresh weight

TTA was determined using a 794 Basic Titrino titrometer (Metrohm, Switzerland) calibrated at 25 °C using 2 g of fruit homogenate diluted into 50 mL deionized water from an ELGA PURELAB Option-R7 water deionizer (ELGA Labwater, UK). Fruit homogenate was used instead of fruit supernatant as significantly lower TTA values were obtained when fruit supernatant initially was used. Initial pH was also determined by the same titrometer before addition of titrant.

SSC was determined using a PR-101α digital refractometer (Atago, Japan). The fruit homogenate was centrifuged in a Jouan B4i centrifuge (Thermo Scientific, USA) at 3800 rpm for 20 minutes to create a supernatant. The refractometer was calibrated at 25 °C and blanked with deionized water and wiped before a drop of the supernatant was placed on the refractometer lens and measured three consecutive times. The measurement was presented as °Brix (% of total dissolved solids). The lens was washed with deionized water between each different supernatant sample.

3.6.2 Vitamin C

Vitamin C is an antioxidant and is therefore a reducing agent. The concentration of Vitamin C in fruits can subsequently be determined by redox titration with a suitable oxidant, such as 2,6-Dichloroindophenol (DCP), which has the capability of quite specifically oxidizing vitamin C (Advancer Instructional Systems, Inc. and the University of California, Santa Cruz, 2011) and is based on the ISO 6557-2 (1984) method utilizing DCP. DCP in its oxidized form is strongly colored, displaying a dark blue color at neutral and alkaline conditions and red color in acidic ones. When DCP is reduced it is colorless making it a good titrant for the reaction. The titration is performed in an acidic environment provided by oxalic acid and after the equivalence point is reached, the reaction mixture will turn pink owing to the additional DCP added in the acidic environment. Oxidized vitamin C has a brown tinge but as the relative concentration and color is far inferior to that of DCP, this contribution is negligible. The redox reaction between vitamin C and DCP is shown in Figure 4.



Figure 8. Redox reaction between L-Ascorbic acid and 2,6-Dichloroindophenol with a stoichiometric relationship of 1:1 made in ChemDraw.

The concentration of vitamin C can then be calculated by redox titration by assuming equal concentration between vitamin C and DCP at equivalence point using Equation 6. The constant 176.12 is the molecular weight of Vitamin C used to convert molar concentration to g L⁻¹ and the constant 1000 is a conversion factor from g L⁻¹ to mgL⁻¹. A

factor of 10 is added in the denominator as a conversion factor from mg L⁻¹ to mg100mL⁻¹.

$$C_{Vit.C} = \frac{V_{DCP} x M_{DCP} x 176.12 x 1000}{\left(\frac{V_{S}}{\left(\frac{V_{Ex}+V_{S}}{V_{S}}\right)}\right) x 10}$$

Equation 6.

- $C_{Vit. C}$ = Concentration of vitamin C (mg 100mL⁻¹)
- V_{DCP} = Volume DCP (mL)

 M_{DCP} = Concentration DCP (M)

 V_s = Volume sample (mL)

 V_{Ex} = Volume extraction solution (mL)

To prepare the DCP titrant 330 mg 2,6-dichloroindophenol sodium salt hydrate and 100 mg sodium bicarbonate were dissolved in 250 mL deionized water with a magnetic stirrer and heater ARE Hot Plate Stirrer (VELP Scientifica, Italy) at 50 °C - 60 °C equating to a concentration of approx. 6.602 x 10⁻⁴ M DCP. DCP is not stable in solution over time and subsequently the titer of the DCP titrant was determined with a vitamin C standard solution. The vitamin C standard used had a concentration of 0.5 g L⁻¹. 50 mL extraction solution was used for each titration consisting of 28 g oxalic acid (2%w/w) in 1 L deionized water. The fruit supernatant sample volume used was 3 mL. Supernatant from homogenized fruit samples was used instead of homogenate to prevent the possible release of vitamin C that may reside in the fruit pulp during titration, as the pink end point of the DCP titrant is only stable for approximately 30 seconds (Advancer Instructional Systems, Inc. and the University of California, Santa Cruz, 2011). The extraction solution and sample were poured into an erlenmeyer beaker and placed on the magnetic stirrer whilst the DCP titrant was added with a digital pipette Multipipette E3x (Eppendorf, USA). The endpoint was established when the solution turned a slight hue of pink. For better contrast, a white piece of paper was placed between the erlenmeyer beaker and the magnetic stirrer.

3.6.3 Pigments

Pigment concentrations were determined spectrophotometrically. To determine the concentration of each pigment spectrophotometrically, the pigments need to be extracted and diluted in solvent to adhere to Beer Lambert's law of linear correlation between absorption and concentration. Organic solvents are necessary to extract both chlorophyll and carotenoid pigments and the use of different solvents alters the absorption maxima of the extracted pigments. Depending on the degree of polarity of solvent used, the absorption maxima of both chlorophylls and carotenoids shift. It is therefore necessary to ascertain these altered maxima for the applicable solvent(s) to increase the accuracy of measurement (Lichtenthaler and Buschmann, 2001).

Thawed fruit homogenate samples were weighed to 2 g and diluted into 8.08 mL 100% acetone for pigment extraction, approximating 20% water and 80% acetone by volume. The samples were subsequently mixed using a Vortex-Genie 2 vortex mixer (Scientific Industries, USA) and stored for 12 hours in darkness at 4 °C for pigment extraction. Afterwards the samples were centrifuged and 3800 rpm for 10 minutes and 200 µL supernatant was transferred into a 96-well microplate with three replicates for each sample. Since the solvent used for extracting pigments is acetone and homogenized cucumber was used, constituting mainly water, wavelengths corresponding to the relative maxima of 20% water in 80% acetone by volume will be used. Beer Lambert's law is only applicable to single pigments and as evident from Figure 3, the absorption integrals of chlorophyll a and b overlap to some extent, making it necessary to subtract a certain absorption value from both chlorophylls. Lichtenthaler and Buschmann (2001) have determined the appropriate wavelengths and constants for measuring absorption and correctly determining respective concentrations of chlorophyll a and b in μ g mL⁻¹ in Equations 3 and 4. Since carotenoids constitute multiple pigments such as xanthines and carotenes, an estimation of their total concentration can be calculated once the concentration of both chlorophyll a and b has been determined. Assuming an absorption maxima at 470 nm for carotenoids, the contribution of chlorophyll *a* and *b* concentration can be subtracted and Lichtenthaler and Buschmann (2001) have determined the correct constants for total carotenoid concentration in μ g/mL in Equation 5. Absorbance should also be in the range 0.3-0.85 for accurate pigment quantification and dilution factor should therefore be adjusted to account for this.

 $\begin{array}{ll} C_a = 12.25 \ x \ A_{663,2} - 2.79 \ x \ A_{646,8} & \mbox{Equation 3. (Lichtenthaler and Buschmann, 2001)} \\ C_b = 21.50 \ x \ A_{646,8} - 5.10 \ x \ A_{663,2} & \mbox{Equation 4. (Lichtenthaler and Buschmann, 2001)} \\ C_{(x+c)} = \frac{(1000 \ x \ A_{470} - 1.82 \ x \ C_a - 85.02 \ x \ C_b)}{198} & \mbox{Equation 5. (Lichtenthaler and Buschmann, 2001)} \\ C_a = \mbox{Concentration of chlorophyll } a \ (\mu g \ mL^{-1}) \\ C_b = \mbox{Concentration of chlorophyll } b \ (\mu g \ mL^{-1}) \\ C_{(x+c)} = \mbox{Concentration of total carotenoids } (\mu g \ mL^{-1}) \\ A_{646,8} = \mbox{Absorbance at 646.8 nm} \\ A_{663,2} = \mbox{Absorbance at 663.2 nm} \\ A_{470} = \mbox{Absorbance at 470 nm} \end{array}$

In accordance with Lichtenhaler and Buschmann, (2001), absorbance was measured at 470, 647 and 663 nm at 22 °C with a spectrophotometer Multiskan Go (Thermo Scientific, USA) using the software Skanlt RE ver. 5.0 (Thermo Scientific, USA) and absorbance values were blank subtracted. Pigment concentration was calculated using Equation 3., 4. and 5. for respective pigments. Equations 3., 4. and 5. are based on Beer Lambert's law and are based on a standard pathlength of 1 cm. When measuring in microwell plates deviating from the 1 cm standard, a pathway correction factor needs be determined by measuring absorbance of the same solvent composition at the same wavelengths with both the microplate wells and 1 cm standard cuvettes prior to concentration calculation. The difference in absorbance constituting the pathway corrected absorbance results were adjusted using Equation 6.

$$A_{P.corr.} = \frac{A_w}{0.82}$$

Equation 6.

 $A_{P.corr.}$ = Pathway corrected absorbance A_w = Absorbance in well

3.6.4 Ionic content

The ionic content of the cucumber samples was measured at 22 °C using a 883 Basic IC plus ion chromatograph (Metrohm, Switzerland) linked to a conductivity detector, a 863 compact autosampler (Metrohm, Switzerland) and a ESA CH-150 column heater (Analytical Instruments LLC, USA) set at 30 °C. For the determination of the anions Cl-, NO₃⁻, PO₄³⁻ and SO₄²⁻, suppressed IC was used using a MSM A ion suppressor (Metrohm, Switzerland) with a 0.1 M sulfuric acid suppressing solution for suppression regeneration and deionized water for suppressor rinsing. Anions were separated using a Metrosep A Supp 5 - 150 x 4 mm column (Metrohm, Switzerland) protected by a Metrosep A Supp 5 – 5 x 4 mm guard column (Metrohm, Switzerland) using an eluent consisting of 3.2 mM sodium carbonate, 1.0 mM sodium bicarbonate and 5% v/v acetone in deionized water. The cations Na⁺, NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ were separated with a Metrosep C4 – 150 x 4 mm column (Metrohm, Switzerland) protected by a Metrosep C4 – 5 x 4 mm guard column (Metrohm, Switzerland) using an eluent consisting of 1.7 mM nitric acid and 0.7 mM 2,6-pyridinedicarboxylic acid in deionized water. Both the eluents for the anions and cations were filtered using 0.45 µm nylon filter membranes (VWR, USA) prior to use.

For the anion sample preparation, the thawed supernatant of each sample was shaken in a vortex mixer before being diluted 30-fold in deionized and filtered water through 0.45 µm nylon syringe filters (VWR, USA). For the cation sample preparation, the same method was used differing only in a sample dilution factor of 50-fold in deionized water.

The different dilution factors reflect the necessity of the ionic concentration of the samples being within in the concentration range of the respective anion and cation standards for accurate concentration measurements. Blank samples were also analyzed to perform blank subtraction of the ionic concentration contribution from the syringe filters. Identification and quantification of the ionic content in the samples were ascertained using external calibration with the Multi Anion Standard 1 for IC (Sigma-Aldrich, Germany) and the Multi Cation Standard SS-2877S (Spectrascan, Sweden) standard solutions ($r^2 > 0.99$, relative standard deviation < 6.5%) using MagIC Net ver 3.2 software (Metrohm, Switzerland). Ionic concentration results were given by the software in ppm equaling mg L⁻¹. Blank subtraction and recalculation to adjusted sample concentrations in mM were further calculated using Equation 9.

$$C_{ax} = \frac{(C_x - C_b) x \, d.factor}{Mw(x)}$$
 Equation 7.

 C_{ax} = Adjusted ion concentration of x ionic species (mM) C_x = lon concentration given of x ionic species by software (mg L⁻¹) d. factor = Dilution factor of sample Mw(x) = Molecular weight of ionic species (g mol⁻¹)

3.7 Statistical methods

For the physical variables a two-way analysis of variance (ANOVA) was performed on the IMEA and DeeRect cultivars separately when freshly harvested and after 4 weeks storage. Light treatment and storage were analyzed as main effects to examine the sole effect of these variables on the cucumber fruits and with the interaction effect (Light*storage) to examine whether different light treatments and storage affected each other. A Tukey HSD post-hoc test was performed for each light treatment with corresponding storage to examine which treatment(s) were potentially significantly different. A one-way ANOVA was also performed between the IMEA and DeeRect cultivars on the independent cultivar variable to determine potential significant difference on the dependent physical variables. This method was also applied to the chemical variables with the exemption of vitamin C, as all samples were fresh in which a one-way ANOVA was performed on the IMEA and DeeRect cultivars separately on the light variable and another one-way ANOVA was performed on the cultivar variable. For the taste test a one-way ANOVA was performed for the independent light variable on perceived taste. The software SPSS Statistics ver. 26 was used to compute the data results.

4. Results

4.1 Physical measurements

Examples of typical cucumbers from the IMEA and DeeRect cultivars subjected to different light treatments after 4 weeks of storage are depicted in Figure 9.



Figure 9. Cucumbers from the cultivars IMEA (left) and DeeRect (right) with exemplary physical characteristics. Each cultivar was subjected to four different light treatments representing the four different cucumbers in each picture namely from right to left: overhead LED, overhead LED + intracanopy LED, overhead HPS + intracanopy LED and overhead HPS.

Table 7 and 8 present the results for all physical variables measured for the IMEA and DeeRect cultivars respectively for each light treatment, both when freshly harvested and when stored for 4 weeks.

Table 7. Results of physical measurements (mean \pm standard deviation) of respective physical variables treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, both fresh n = 6 and stored for 4 weeks n = 6 for the cultivar IMEA. Δ Storage (%) signifies the relative change between fresh and stored cucumbers for respective variables in %. A two-way ANOVA was performed between light and storage variables. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between each different light treatment and corresponding storage indicated by different letters.

| Cultivar | Light | Storage | Length (cm) | Diameter (cm) | Weight (g) | Color (scale 5-9) |
|---------------|-------|---------|---------------|---------------|----------------|-------------------|
| | | | | | | |
| IMEA | LED | Fresh | 29.1 ± 1.3abc | 3.9 ± 0.2abc | 302.4 ± 6.9cd | 8.0 ± 0.0c |
| | | 4 weeks | 28.8 ± 1.2abc | 3.7 ± 0.1ab | 266.1 ± 7.5ab | 6.8 ± 0.4ab |
| | LED+L | Fresh | 27.9 ± 0.4ab | 4.0 ± 0.1bc | 301.7 ± 7.8cd | 8.0 ± 0.0c |
| | | 4 weeks | 28.0 ± 0.7ab | 3.7 ± 0.0ab | 263.7 ± 7.9ab | 7.5 ± 0.6bc |
| | HPS+L | Fresh | 30.6 ± 2.0c | 4.1 ± 0.1c | 319.6 ± 17.1d | 8.0 ± 0.0c |
| | | 4 weeks | 30.2 ± 1.9bc | 3.7 ± 0.1ab | 283.7 ± 13.5bc | 7.7 ± 0.5bc |
| | HPS | Fresh | 28.7 ± 1.2abc | 3.9 ± 0.4ab | 297.3 ± 14.6c | 7.7 ± 0.5bc |
| | | 4 weeks | 27.6 ± 0.9a | 3.7 ± 0.1a | 248.9 ± 12.1a | 6.2 ± 1.0a |
| Δ Storage (%) | | | -1.5 | -7.3 | -13.0 | -11.1 |
| Light | | | *** | ns | *** | *** |
| Storage | | | ns | *** | *** | *** |
| Light*Storage | | | ns | ns | ns | ** |
| | | | | | | |

Table 8. Results of physical measurements (mean ± standard deviation) of respective physical variables treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, both fresh n = 6 and stored for 4 weeks n = 6 for the cultivar DeeRect. Δ Storage (%) signifies the relative change between fresh and stored cucumbers for respective variables in %. A two-way ANOVA was performed between light and storage variables. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between each different light treatment and corresponding storage indicated by different letters.

| Cultivar | Light | Storage | Length (cm) | Diameter (cm) | Weight (g) | Color (scale 5-9) |
|---------------|-------|---------|-------------|---------------|-----------------|-------------------|
| | | | | | | |
| DeeRect | LED | Fresh | 30.9 ± 0.6a | 4.0 ± 0.0c | 345.5 ± 50.9c | 8.5 ± 0.6b |
| | | 4 weeks | 29.9 ± 0.8a | 3.7 ± 0.1a | 257.4 ± 17.7a | 7.7 ± 0.5ab |
| | LED+L | Fresh | 31.1 ± 0.8a | 4.0 ± 0.1bc | 310.9 ± 6.7abc | 8.2 ± 0.4ab |
| | | 4 weeks | 29.7 ± 0.9a | 3.7 ± 0.2ab | 257.1 ± 28.5a | 7.8 ± 0.4ab |
| | HPS+L | Fresh | 31.0 ± 1.4a | 4.0 ± 0.2c | 324.5 ± 28.1bc | 8.3 ± 0.5ab |
| | | 4 weeks | 30.7 ± 1.2a | 3.8 ± 0.3abc | 263.9 ± 66.2ab | 7.5 ± 0.6a |
| | HPS | Fresh | 31.8 ± 1.7a | 3.9 ± 0.1abc | 318.3 ± 19.7abc | 8.0 ± 0.0ab |
| | | 4 weeks | 30.9 ± 1.7a | 3.6 ± 0.1a | 270.4 ± 17.0ab | 7.7 ± 0.5ab |
| Δ Storage (%) | | | -2.9 | -7.1 | -19.3 | -7.1 |
| Light | | | ns | ns | ns | ns |
| Storage | | | * | *** | *** | *** |
| Light*Storage | | | ns | ns | ns | ns |
| | | | | | | |

Table 9 presents the data for all physical variables measured in Table 7 and 8 with regards to the difference between cultivars IMEA and DeeRect.

Table 9. Results of physical measurements (mean \pm standard deviation) for the cultivars IMEA and DeeRect with n = 48 samples for each cultivar. A one-way ANOVA was performed for the cultivar variable with significance indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001.

| Cultivar | Length (cm) | Diameter (cm) | Weight (g) | Color (scale 5-9) |
|----------|-------------|---------------|--------------|-------------------|
| IMEA | 28.9 ± 1.6 | 3.8 ± 0.2 | 285.4 ± 25.1 | 7.5 ± 0.8 |
| DeeRect | 30.7 ± 1.3 | 3.8 ± 0.2 | 293.5 ± 46.1 | 8.0 ± 0.5 |
| Cultivar | *** | ns | ns | *** |

4.1.1 Length

There was a clear significant difference between the overall length of the IMEA cultivar compared to the DeeRect cultivar with an average DeeRect cultivar cucumber measuring 30.7 cm and an average IMEA cucumber cultivar measuring 28.85 cm (Table 9). The two-way ANOVA conducted on the IMEA and DeeRect cultivars showed that light treatment was significant for the IMEA cultivar with the light treatment overhead HPS combined with intracanopy LED producing the longest cucumbers and the light treatment overhead LED combined with intracanopy LED producing the shortest cucumbers (Table 7). Light treatment was however not significant for the DeeRect cultivar, but storage was in which the stored cucumbers had consistently shorter lengths than the fresh ones on average (Table 8), though not significant enough for the Tukey test to place into a separate homogeneous subset.

4.1.2 Diameter

No significant difference between the cultivars IMEA and DeeRect was found with regards to diameter. Light treatment was also not significant for both IMEA and DeeRect cultivars, but both cultivars had a significantly smaller diameter after storage (Table 7 and 8).

4.1.3 Weight

The cultivars IMEA and DeeRect showed no significant difference in terms of weight between the cultivars. For the IMEA cultivar light treatment was clearly significant with the light treatment overhead HPS in combination with intracanopy LED producing substantially heavier cucumber fruits than the other light treatments (Table 7). On the other hand, no significant difference was found in the DeeRect cultivar with regards to light treatment, but both cultivars were significantly lighter after storage with an average decrease in weight for IMEA at 13.0% and 19.3% for DeeRect (Table 7 and 8). Figure 10 illustrates the fairly homogenous decrease for both the IMEA and DeeRect cultivars as a response to different light treatments. This is also reflected in the results from Table 7 and 8 in which storage*light was not significant for either cultivar.



Figure 10. Bar graph illustrating weight (g) of cucumber from the cultivars IMEA and DeeRect subjected to the different light treatments overhead LED, overhead LED in combination with intracanopy LED, overhead HPS in combination with intracanopy LED and overhead HPS both freshly harvested and after 4 weeks of storage. Error bars represent 95% confidence intervals.

4.1.4 Color

There was a significant difference between the IMEA and DeeRect cultivars with regards to perceived color with the DeeRect cultivar averaging at 7.96 compared to IMEA at 7.48 on the color scale (Table 9). For the IMEA cultivar light treatment was significant for perceived color with both the light treatments overhead LED in combination with intracanopy LED and overhead HPS in combination with intracanopy LED producing greener colored cucumbers (Table 7). Interestingly light*storage was significant for perceived color for IMEA and the light treatments overhead LED in combination with intracanopy LED and overhead HPS in combination with intracanopy LED showed a smaller difference in perceived color values than the rest of the light treatments as shown in Figure 10. The DeeRect cultivar did not show significantly different values in response to different light treatments or in response to light*storage. Storage was however significant for both IMEA and DeeRect, showing a decline in perceived greenness after storage (Table 7 and 8).



Figure 10. Bar graph depicting average perceived greenness on a color scale from 5-9 of cucumbers from the IMEA cultivar in response to the light treatments overhead LED, overhead LED in combination with intracanopy LED, overhead HPS in combination with intracanopy LED and overhead HPS both freshly harvested and after 4 weeks of storage. Error bars represent 95% confidence intervals.

4.2 Chemical measurements

The results for the chemical measurements DMC, SSC, pH, TTA and pigment concentrations are presented in Table 10 and 11 for the IMEA and DeeRect cultivars respectively for each light treatment, both when freshly harvested and when stored for 4 weeks.

Table 10. Results of chemical measurements (mean \pm standard deviation) of respective chemical variables treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, both fresh n = 3 and stored for 4 weeks n = 6 for the cultivar IMEA. Δ Storage (%) signifies the relative change between fresh and stored cucumbers for respective variables in %. A two-way ANOVA was performed between light and storage variables. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between each different light treatment and corresponding storage indicated by different letters.

| Cultivar | Light | Storage | DMC (%) | SSC (°Brix) | рН | TTA (% MAE FW) | TTA/SSC | Chlorophyll a + b (µg mL ^{.1}) | Total carotenoids (μg mL ^{.1}) |
|---------------|-------|---------|----------------|---------------|--------------|----------------|-----------------|---|--|
| IMEA | LED | Fresh | 2.71 ± 0.17abc | 2.32 ± 0.17ab | 5.98 ± 0.05a | 0.079 ± 0.008a | 0.034 ± 0.002ab | 5.04 ± 0.56abc | 0.57 ± 0.01a |
| | | 4 weeks | 2.74 ± 0.17abc | 2.27 ± 0.12ab | 6.1 ± 0.06a | 0.080 ± 0.009a | 0.036 ± 0.005ab | 3.28 ± 0.1.27ab | 0.49 ± 0.17a |
| | LED+L | Fresh | 2.91 ± 0.17bc | 2.29 ± 0.05ab | 6.02 ± 0.03a | 0.072 ± 0.002a | 0.032 ± 0.001ab | 5.71 ± 1.29bc | 0.61 ± 0.20a |
| | | 4 weeks | 2.72 ± 0.10abc | 2.21 ± 0.04ab | 6.03 ± 0.15a | 0.085 ± 0.005a | 0.038 ± 0.002ab | 2.88 ± 0.40a | 0.40 ± 0.05a |
| | HPS+L | Fresh | 2.98 ± 0.12c | 2.48 ± 0.25b | 6.10 ± 0.03a | 0.068 ± 0.004a | 0.028 ± 0.001a | 6.45 ± 1.16c | 0.67 ± 0.23a |
| | | 4 weeks | 2.79 ± 0.12bc | 2.32 ± 0.14ab | 6.04 ± 0.13a | 0.087 ± 0.011a | 0.038 ± 0.006ab | 4.38 ± 1.69abc | 0.47 ± 0.12a |
| | HPS | Fresh | 2.46 ± 0.13a | 2.09 ± 0.15a | 5.94 ± 0.08a | 0.076 ± 0.003a | 0.037 ± 0.001ab | 5.13 ± 0.40abc | 0.54 ± 0.11a |
| | | 4 weeks | 2.59 ± 0.15ab | 2.18 ± 0.04a | 5.97 ± 0.43a | 0.085 ± 0.015a | 0.039 ± 0.008b | 3.95 ± 1.40abc | 0.46 ± 0.15a |
| Δ Storage (%) | | | -1.9 | -2.2 | 0.4 | 12.5 | 14.1 | -35.2 | -23.6 |
| Light | | | *** | *** | ns | ns | ns | ns | ns |
| Storage | | | ns | ns | ns | ** | ** | *** | ** |
| Light*Storage | | | ns | ns | ns | ns | ns | ns | ns |

Table 11. Results of chemical measurements (mean \pm standard deviation) of respective chemical variables treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, both fresh n = 3 and stored for 4 weeks n = 6 for the cultivar DeeRect. Δ Storage (%) signifies the relative change between fresh and stored cucumbers for respective variables in %. A two-way ANOVA was performed between light and storage variables. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between each different light treatment and corresponding storage indicated by different letters.

| Cultivar | Light | Storage | DMC (%) | SSC (°Brix) | рН | TTA (% MAE FW) | TTA/SSC | Chlorophyll <i>a</i> + <i>b</i> (µg mL ⁻¹) | Total carotenoids (μg mL ⁻¹) |
|-------------------|-------|---------|--------------|--------------|--------------|----------------|----------------|---|---|
| DeeRect | LED | Fresh | 2.98 ± 0.27a | 2.66 ± 0.05a | 6.34 ± 0.22a | 0.050 ± 0.007a | 0.019 ± 0.003a | 7.45 ± 1.55b | 0.78 ± 0.20ab |
| | | 4 weeks | 3.25 ± 0.24a | 2.85 ± 0.24a | 5.96 ± 0.50a | 0.092 ± 0.016b | 0.032 ± 0.005b | 4.81 ± 1.12ab | 0.86 ± 0.13ab |
| | LED+L | Fresh | 3.30 ± 0.24a | 2.84 ± 0.17a | 6.23 ± 0.40a | 0.048 ± 0.005a | 0.017 ± 0.003a | 6.37 ± 2.98ab | 0.74 ± 0.38ab |
| | | 4 weeks | 3.20 ± 0.25a | 2.71 ± 0.18a | 6.17 ± 0.11a | 0.078 ± 0.007b | 0.029 ± 0.003b | 4.74 ± 1.52ab | 0.79 ± 0.23ab |
| | HPS+L | Fresh | 3.37 ± 0.29a | 2.87 ± 0.23a | 6.58 ± 0.26a | 0.047 ± 0.008a | 0.016 ± 0.003a | 6.72 ± 1.82ab | 0.58 ± 0.30ab |
| | | 4 weeks | 3.38 ± 0.27a | 2.61 ± 0.22a | 5.94 ± 0.43a | 0.076 ± 0.005b | 0.029 ± 0.003b | 3.25 ± 1.29a | 0.64 ± 0.22ab |
| | HPS | Fresh | 3.22 ± 0.18a | 2.54 ± 0.12a | 6.42 ± 0.10a | 0.051 ± 0.006a | 0.020 ± 0.003a | 6.20 ± 1.04ab | 0.39 ± 0.10a |
| | | 4 weeks | 3.41 ± 0.19a | 2.64 ± 0.20a | 6.14 ± 0.06a | 0.083 ± 0.008b | 0.031 ± 0.002b | 6.18 ± 1.54ab | 1.09 ± 0.21b |
| Δ Storage (%) | | | 2.8 | -0.1 | 5.4 | 40.7 | 40.9 | -29.1 | 26.3 |
| Light | | | ns | ns | ns | ns | ns | ns | ns |
| Storage | | | ns | ns | ** | *** | *** | ** | ** |
| Light *Storage | | | ns | ns | ns | ns | ns | ns | * |

Table 12 presents the data for all the chemical variables given in Table 10 and 11 with regards to the difference between cultivars IMEA and DeeRect.

Table 12. Results of chemical measurements (mean \pm standard deviation) for the cultivars IMEA and DeeRect with n = 36 for each cultivar. A one-way ANOVA was performed for the cultivar variable with significance indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001.

| Cultivar | DMC (%) | SSC (°Brix) | рН | TTA (% MAE FW) | TTA/SSC | Chlorophyll a + b (µg mL ^{.1}) | Total carotenoids (µg mL⁻¹) |
|----------|-------------|-------------|-------------|-------------------|-------------------|---|-----------------------------------|
| IMEA | 2.73 ± 0.19 | 2.26 ± 0.14 | 6.03 ± 0.19 | 0.081 ± 0.010 | 0.036 ± 0.005 | 4.27 ± 1.54 | 0.50 ± 0.15 |
| DeeRect | 3.28 ± 0.25 | 2.71 ± 0.21 | 6.17 ± 0.35 | 0.071 ± 0.019 | 0.026 ± 0.007 | 5.39 ± 1.91 | 0.77 ± 0.28 |
| Cultivar | *** | *** | * | ** | *** | ** | *** |

4.2.1 DMC

There was a significance difference in DMC between the IMEA and DeeRect cultivars where DeeRect had an average DMC of 3.28% whilst IMEA had a DMC of 2.73% on average (Table 12). The IMEA cultivar showed a significant difference in terms of DMC when subjected to different light treatments in which the light treatment overhead HPS produced cucumbers with significantly lower DMC (Table 10). The DeeRect cultivar showed no significance in terms of light treatment and neither IMEA nor DeeRect cultivars showed significant differences with regards to storage.

4.2.2 SSC

Similar findings to the DMC results were found in SSC content. A significant difference between the IMEA and DeeRect cultivars was demonstrated with respective SSC contents of 2.26 °Brix and 2.71 °Brix (Table 12). For the IMEA cultivar light treatment showed a significant difference with the light treatment overhead HPS in combination with intracanopy LED producing cucumbers with high amounts of SSC and the light treatment overhead HPS producing cucumbers with the lowest amount of SSC (Table 10). The DeeRect cultivar showed no significant difference in SSC concentration with regards to different light treatments and both DeeRect and IMEA cultivars showed no significant difference in SSC concentration as a function of storage.

4.2.3 pH, TTA and TTA/SSC

A significant difference was found in terms of measured pH and TTA concentration between the IMEA and DeeRect cultivars with the DeeRect cultivar averaging at pH 6.17 and a TTA concentration of 0.071 % MAE whilst the IMEA cultivar averaged at pH 6.03 and a TTA concentration of 0.081 % MAE (Table 12). Light treatment was not significant for both DeeRect and IMEA cultivars, but both responded to storage with regards to pH and TTA concentration with subsequent lower pH value and higher TTA concentration after storage (Table 10 and 11). Storage was however not significant enough for the Tukey test to place TTA in the IMEA cultivar into a separate homogeneous subset, but clearly did for the DeeRect cultivar. Similar results were found regarding the proportion TTA/SSC, in which the DeeRect cultivar had an average proportion of 0.026 as opposed to the IMEA cultivars the proportion shows a significant increase in response to storage, though again the Tukey test shows a more pronounced difference for the DeeRect cultivar, but no significant difference in terms of light treatment (Table 10 and 11).

4.2.4 Pigments

There was a significant difference between the IMEA and DeeRect cultivars with regards to both chlorophyll a + b content and total carotenoid content. The IMEA cultivar had an average chlorophyll a + b content of 4.27 µg mL⁻¹ and an average total carotenoid content of 0.5 µg mL⁻¹ (Table 12). The DeeRect cultivar on the other hand had an average chlorophyll a + b content of 5.39 µg mL⁻¹ and an average total carotenoid content of 0.77 µg mL⁻¹ (Table 12). For both the IMEA and DeeRect cultivar, light

treatment did not have a significant effect on chlorophyll a + b and total carotenoid content but storage did. For the IMEA cultivar both chlorophyll a + b content and total carotenoid content declined significantly in response to storage, though the not enough for the Tukey test to place total carotenoids into a separate homogeneous subset (Table 10). For the DeeRect cultivar however chlorophyll a + b content declined as a response to storage, but total carotenoid content increased (Table 11). Storage*light was also significant for total carotenoid content in the DeeRect cultivar with the light treatment overhead HPS having a markedly higher total carotenoid concentration after storage than the other light treatments, shown in Figure 11.



Figure 11. Bar graph depicting average total carotenoid content (µg mL⁻¹) of cucumbers from the DeeRect cultivar in response to the light treatments overhead LED, overhead LED in combination with intracanopy LED, overhead HPS in combination with intracanopy LED and overhead HPS both freshly harvested and after 4 weeks of storage. Error bars represent 95% confidence intervals.

4.2.5 Vitamin C

Vitamin C measurements for both the IMEA and DeeRect cultivars subjected to different light treatments when fresh, along with the difference in vitamin C concentration between the cultivars IMEA and DeeRect is presented in Table. 13.

Table 13. Vitamin C concentration (mg 100mL⁻¹) of cucumbers (mean ± standard deviation) treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, for the cultivars IMEA and DeeRect n = 3. The overall difference between the cultivars IMEA and DeeRect are also presented with n = 12 for each cultivar. A one-way ANOVA was performed both for the light variable for each separate cultivar and for the cultivar variable. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between different light treatments indicated by different letters.

| Cultivar | Light | Vitamin C (mg 100mL ⁻¹) |
|----------|-------|-------------------------------------|
| | | |
| IMEA | LED | 8.45 ± 0.40a |
| | | |
| | LED+L | 9.36 ± 1.11ab |
| | | |
| | HPS+L | 10.57 ± 0.51b |
| | | |
| | HPS | 8.86 ± 0.62ab |
| | | |
| | Light | * |
| | | |
| DeeRect | LED | 13.19 ± 1.11a |
| | | 14 50 1 0 50 |
| | LED+L | 11.53 ± 0.50a |
| | | 10.40 ± 9.065 |
| | NF3TL | 19.49 ± 0.00a |
| | HPS | 13 24 + 2 72a |
| | | 10.24 2 2.720 |
| | Light | ns |
| | | |
| IMEA | | 9.31 ± 1.03 |
| DeeRect | | 14.36 ± 4.85 |
| Cultivar | | ** |
| | | |

A significant difference was seen in vitamin C content between the cultivars IMEA and DeeRect with the IMEA cultivars averaging at 9.31 mg 100 mL⁻¹ and the DeeRect cultivar averaging at 14.36 mg 100 mL⁻¹ (Table 13). The IMEA cultivar also showed significant difference in vitamin C contents in terms of different light treatments with the light treatment overhead HPS in combination with intracanopy LED producing cucumbers with the highest relative vitamin C concentrations and the light treatment overhead LED producing cucumbers with the lowest relative vitamin C concentrations (Table 13). The DeeRect cultivar showed no significance between different light treatments despite the light treatment overhead HPS in combination with intracanopy LED producing cucumbers due to a high standard deviation of the former light treatment (Table 13).

4.2.6 Ionic content

The concentration of the ionic species Cl⁻, NO₃⁻, PO₄³⁻ and SO₄²⁻, Na⁺, NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ of the cultivar IMEA are presented in Table 14. The concentration of the ionic species Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻, Na⁺, NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ for the DeeRect cultivar are presented in Table 15. Concentrations are given respectively for each light treatment, both when freshly harvested and when stored for 4 weeks for both cultivars.

Table 14. Ionic concentration (mM) of cucumbers (mean \pm standard deviation) of respective ionic species treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, both fresh and stored for 4 weeks for the cultivar IMEA. For the ions n = 3 for the fresh samples and n = 6 for the stored samples, except for n = 2 for the cations with overhead LED light treatment. Δ Storage (%) signifies the relative change between fresh and stored cucumbers for respective variables in %. – denotes average values that fell below the detection threshold. A two-way ANOVA was performed between light and storage variables. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between each different light treatment and corresponding storage indicated by different letters.

| Cultivar | Light | Storage | Cl ⁻ (mM) | NO₃⁻ (mM) | PO₄³ (mM) | SO ₄ ²⁻ (mM) | Na⁺ (mM) | NH₄⁺ (mM) | K⁺ (mM) | Ca²+ (mM) | Mg²+ (mM) |
|---------------|-------|---------|----------------------|---------------|----------------|------------------------------------|--------------|--------------|---------------|--------------|--------------|
| | | | | | | | | | | | |
| IMEA | LED | Fresh | 0.99 ± 0.13ab | 6.07 ± 0.37ab | 7.82 ± 0.89a | 1.75 ± 0.26a | 0.02 ± 0.03a | 0.32 ± 0.01a | 47.40 ± 3.91a | 5.37 ± 0.08a | 3.68 ± 0.25a |
| | | 4 weeks | 1.37 ± 0.21bc | 3.40 ± 1.67a | 9.16 ± 1.01abc | 2.26 ± 0.15b | 0.03 ± 0.02a | 0.19 ± 0.02a | 45.69 ±3.30a | 3.69 ± 2.18a | 4.01 ± 0.35a |
| | LED+L | Fresh | 0.80 ± 0.02a | 4.45 ± 1.09ab | 8.28 ± 0.07ab | 1.78 ± 0.14a | 0.08 ± 0.17a | 0.25 ± 0.07a | 42.36 ± 1.76a | 3.86 ± 0.16a | 2.96 ± 0.14a |
| | | 4 weeks | 1.15 ± 0.26abc | 5.79 ± 2.65ab | 10.10 ± 1.05bc | 2.19 ± 0.12b | 0.00 ± 0.03a | 0.16 ± 0.04a | 51.69 ± 6.91a | 4.04 ± 0.58a | 3.62 ± 0.40a |
| | HPS+L | Fresh | 1.45 ± 0.20bc | 4.20 ± 0.20ab | 8.25 ±1.30ab | 1.68 ± 0.17a | 0.20 ± 0.35a | 0.31 ± 0.09a | 46.55 ± 8.67a | 4.96 ± 2.33a | 3.82 ± 1.50a |
| | | 4 weeks | 1.50 ± 0.32c | 4.75 ± 2.59ab | 10.24 ± 0.64c | 2.19 ± 0.18b | 1.44 ± 0.27b | 1.12 ± 0.19b | 49.24 ± 5.54a | 3.40 ± 1.84a | 3.29 ± 0.61a |
| | HPS | Fresh | 1.14 ± 0.13abc | 7.88 ± 1.25ab | 8.49 ±0.41abc | 1.83 ± 0.10a | – ± 0.07a | 0.25 ± 0.04a | 47.71 ± 8.67a | 6.86 ± 1.37a | 4.09 ± 1.10a |
| | | 4 weeks | 1.37 ± 0.08bc | 9.21 ± 3.32b | 9.59 ± 0.48abc | 2.37 ± 0.05b | 1.78 ± 0.67b | 1.06 ± 0.06b | 52.31 ± 5.05a | 5.35 ± 0.91a | 3.96 ± 0.36a |
| Δ Storage (%) | | | 18.9 | 2.4 | 16.0 | 21.9 | 90.6 | 56.0 | 7.7 | -21.6 | 2.3 |
| Light | | | *** | ** | ns | ns | *** | *** | ns | * | ns |
| Storage | | | ** | ns | *** | *** | *** | *** | ns | * | ns |
| Light*Storage | | | ns | ns | ns | ns | *** | *** | ns | ns | ns |

Table 15. Ionic concentration (mM) of cucumbers (mean \pm standard deviation) of respective ionic species treated with different light combinations: LED overhead (LED), overhead LED and intracanopy LED (LED+L), overhead HPS and intracanopy LED (HPS+L) and overhead HPS, both fresh stored for 4 weeks for the cultivar DeeRect. For the ions n = 3 for the fresh samples and n = 6 for the stored samples except for n = 5 for the cations with HPS light treatment. Δ Storage (%) signifies the relative change between fresh and stored cucumbers for respective variables in %. – denotes average values that fell below the detection threshold. A two-way ANOVA was performed between light and storage parameters. Significance is indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. A post-hoc test (Tukey HSD p≤0.05) was performed to examine differences between each different light treatment and corresponding storage indicated by different letters.

| Cultivar | Light | Storage | Cl [.] (mM) | NO₃⁻ (mM) | PO₄³- (mM) | SO ₄ ²⁻ (mM) | Na⁺ (mM) | NH₄⁺ (mM) | K⁺(mM) | Ca²+ (mM) | Mg ²⁺ (mM) |
|-------------------|-------|---------|----------------------|--------------|---------------|------------------------------------|-------------------|---------------|----------------|--------------|-----------------------|
| | | | | | | | | | | | |
| DeeRect | LED | Fresh | 1.59 ± 0.05a | 0.82 ± 0.26a | 6.16 ± 0.58a | 2.00 ± 0.01ab | – ± 0.03ab | 0.02 ± 0.09a | 36.60 ± 2.93a | 2.05 ± 0.05a | 2.32 ± 0.13a |
| | | 4 weeks | 2.46 ± 0.26b | 0.90 ± 0.57a | 8.93 ± 1.08b | 2.81 ± 0.16c | 0.04 ± 0.14ab | 0.67 ± 0.68ab | 49.28 ± 3.51b | 2.33 ± 0.73a | 2.53 ± 0.57a |
| | LED+L | Fresh | 1.63 ± 0.31a | 0.57 ± 0.03a | 7.17 ± 0.76ab | 1.98 ± 0.32ab | – ± 0.05ab | -±0.02a | 39.60 ± 2.08ab | 1.59 ± 0.23a | 2.06 ± 0.26a |
| | | 4 weeks | 2.10 ± 0 34ab | 0.89 ± 0.79a | 9.00 ± 1.39b | 2.67 ± 0.45c | 0.20 ± 0.07 b | 1.30 ± 0.21b | 49.72 ± 7.02b | 2.00 ± 0.47a | 3.06 ± 0.53a |
| | HPS+L | Fresh | 1.55 ± 0.18a | 1.12 ± 0.79a | 7.12 ± 0.63ab | 1.97 ± 0.29ab | 0.01 ± 0.04ab | 0.45 ± 0.24a | 42.46 ± 5.22ab | 2.33 ± 0.65a | 2.12 ± 0.38a |
| | | 4 weeks | 2.10 ± | 0.89 ± 0.78a | 8.43 ± 0.80b | 2.51 ± 0.20bc | – ± 0.04a | 0.03 ± 0.08a | 47.27 ± 4.69ab | 2.36 ± 0.40a | 2.84 ± 0.56a |
| | HPS | Fresh | 1.36 ± 0.24a | 0.93 ± 0.89a | 7.33 ± 0.59ab | 1.84 ± 0.25a | 0.01 ± 0.00ab | 0.24 ± 0.05a | 41.01 ± 3.00ab | 2.12 ± 0.41a | 2.05 ± 0.22a |
| | | 4 weeks | 1.85 ± | 0.97 ± 0.76a | 9.27 ± 0.82b | 2.36 ± 0.16abc | 0.12 ± 0.53b | 0.12 ± 0.07a | 50.13 ± 3.70b | 2.26 ± 0.38a | 2.46 ± 0.38a |
| ∆ Storage (%) | | | 27.9 | 5.5 | 22.0 | 24.8 | 69.8 | 69.8 | 18.6 | 9.4 | 21.8 |
| Light | | | ns | ns | ns | ns | ns | * | ns | ns | ns |
| Storage | | | *** | ns | *** | *** | ns | ** | *** | ns | *** |
| Light* Storage | | | ns | ns | ns | ns | ** | *** | ns | ns | ns |

Table 16 presents the data for the anionic concentrations given in Table. 14 and 15 with regards to the difference between cultivars IMEA and DeeRect.

Table 16. Ionic concentration (mean ± standard deviation) for the cultivars IMEA and DeeRect. For the anionic samples n = 36 and for the cationic samples n = 35. – denotes average values that fell below the detection threshold. A one-way ANOVA was performed for the cultivar variable with significance indicated as ns = not significant, * = $p \le 0.05$, ** = $p \le 0.01$ and *** = $p \le 0.001$.

| Cultivar | CI [.] (mM) | NO₃ ⁻ (mM) | PO₄ ³⁻ (mM) | SO₄²- (mM) | Na⁺ (mM) | NH₄⁺ (mM) | K⁺ (mM) | Ca²⁺ (mM) | Mg²+ (mM) |
|----------|-------------------------|--------------------------|---------------------------|----------------|----------------|----------------|-----------------|----------------|----------------|
| IMEA | 1.26 ± 0.28 | 5.74 ± 2.83 | 9.25 ± 1.12 | 2.09 ± 0.28 | 0.58 ± 0.82 | 0.52 ± 0.43 | 48.52 ± 6.00 | 4.48 ± 1.69 | 3.69 ± 0.67 |
| DeeRect | 1.93 ± 0.45 | 0.89 ± 0.64 | 8.25 ± 1.32 | 2.37 ± 0.41 | - ± 0.29 | 0.42 ± 0.55 | 45.92 ± 6.22 | 2.16 ± 0.49 | 2.53 ± 0.54 |
| Cultivar | *** | *** | *** | *** | *** | ns | ns | *** | *** |

There was a significant difference in all ionic species analyzed between the cultivars IMEA and DeeRect with the exemption of Na⁺ and NH₄⁺ (Table 16). Cl⁻ concentrations were higher in the DeeRect cultivar with an average of 1.93 mM compared to an average of 1.26 mM for the IMEA cultivar (Table 16). The difference in NO₃⁻ concentration was however opposite in which the IMEA cultivar had an average concentration of 5.74 mM compared to the substantially smaller 0.89 mM average for the DeeRect cultivar (Table 16). Likewise, the PO₄³⁻ concentration was greater in the IMEA cultivar cucumbers with an average of 9.25 mM compared to the average concentration of PO₄³⁻ in the DeeRect cultivar at 8.25 mM (Table 16). The concentration of SO₄²⁻ was on the contrary larger in the DeeRect cultivar with an average of 2.37 mM, whilst the IMEA cultivar had an average of 2.09 mM (Table 16). The cucumbers of the IMEA cultivar had consistently higher concentrations of K⁺, Ca²⁺ and Mg²⁺ than those from the DeeRect cultivar (Table 16).

The results for the ionic concentration in the IMEA cultivar were mixed for different anionic species in terms of significance with regards to light treatment and storage variables. For Cl⁻ and NO₃⁻ concentrations light treatment was significant with the light

treatment overhead HPS in combination with intracanopy LED producing cucumbers with the highest concentration of CI⁻ whilst the light treatment overhead LED in combination with intracanopy LED produced cucumbers with the lowest concentration of Cl⁻ (Table 14). With regards to NO₃⁻ concentration, the light treatment overhead HPS produced cucumbers with higher values than all other light treatments (Table 14). The anionic species Cl⁻, PO₄³⁻ and SO₄²⁻ were significant regarding storage, in which all had higher concentrations in stored cucumbers compared to fresh ones (Table 14). For the cationic species in the IMEA cultivar, mixed results in terms of significance towards light treatment and storage were also observed. The cucumbers subjected to both the light treatments including overhead HPS had significantly higher Na⁺ and NH₄⁺ concentrations than the other light treatments (Table 14). Light treatment was also barely significant for Ca²⁺ indicating that the light treatment overhead HPS in combination with intracanopy LED produced cucumbers with higher Ca²⁺ values, though this result was not strong enough for the Tukey test to place it into a separate homogeneous subset (Table 14). Na⁺ and NH₄⁺ for the IMEA cultivar increased significantly in concentration as a response to storage, except for Ca²⁺ which decreased significantly, though again not enough for the Tukey test to place it into a separate homogeneous subset (Table 14).

The results for both the anionic and cationic concentrations in the DeeRect cultivar showed no significance in terms of light treatment with the exception of NH₄⁺ which exhibited a higher concentration for the light treatment overhead LED in combination with intracanopy LED (Table 15). Multiple ionic species for the DeeRect cultivar demonstrated significance concerning the storage variable however, with Cl⁻, PO₄³⁻, SO₄²⁻, NH₄⁺, K⁺, and Mg²⁺ having significantly higher concentrations in stored cucumbers compared to fresh ones (Table 15). Regarding Mg²⁺, this result was again not strong enough for the Tukey test to place it into a separate homogeneous subset.

4.3 Taste test

The results from the two taste tests on fresh cucumbers from the IMEA cultivar subjected to different light treatments are presented in Table 17.

Table 17. Taste test results (mean \pm standard deviation) from two taste tests from a panel of employees at NIBIO Særheim given a questionnaire from (-3) signifying least preference to 3 signifying most preference in rising integers of 1 whole number concerning taste preference on fresh IMEA cultivar cucumbers subjected to the light treatments overhead LED, overhead LED in combination with intracanopy LED, overhead HPS in combination with intracanopy LED and overhead HPS. The total number of subjects for the two taste tests were n = 23. A one-way ANOVA was performed for the light variable with significance indicated as ns = not significant, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001.

| Cultivar | Light | Taste score ((-3) – 3) |
|----------|-------|------------------------|
| IMEA | LED | 1.22 ± 1.09 |
| | LED+L | 1.83 ± 0.89 |
| | HPS+L | 1.00 ± 1.28 |
| | HPS | 1.30 ± 1.22 |
| | Light | ns |

The different light treatments were not found significant for test preference amongst the panel of test subjects at NIBIO Særheim from two taste tests, suggesting marginal if any distinguishable perceived taste difference of commercial value. The cucumbers subjected to the light combination overhead LED in combination with intracanopy LED were attributed the highest taste score but were also linked to a high standard deviation indicating significant variability in taste preference amongst the test subjects.

5. Discussion

5.1 Results of physical parameters

The dimensional parameters length and diameter along with measured weight of cucumbers might be positively correlated to the photosynthetic rate of both the C. sativus plants in addition to photosynthesis of the cucumber fruit itself as Sui et. al., (2017) discovered that approximately 9.4 % of the carbon accumulation in cucumber fruits was derived from photosynthesis of the fruit exocarp which has a similar chlorophyll content to the leaves of the C. sativus plant. Therefore, cucumber fruits can be considered both sink and source organs referring to the fruit's ability to attract assimilates but also synthesize them. Assuming that sink strength is not a limiting factor, an increase in photosynthesis should increase fruit growth (Lemoine et. al., 2013). For the IMEA cultivar the light treatment overhead HPS in combination with intracanopy LED produced the longest and heaviest cucumbers, suggesting an increase in photosynthesis and sink strength in the C. sativus plants and fruits subjected to this light treatment. This observation is also in accordance with the measured PAR values of the overhead HPS lights in combination with intracanopy LED which were the highest combination shown in Table. 4. Storage impacted the weight of both the IMEA and DeeRect cucumbers in particular negatively, which is expected due to water loss through transpiration during storage. No relationship between a certain light treatment and weight loss was found for either cultivar, suggesting that specific light treatments play a negligible role in terms of storage and that conventional means of weight preservation such as plastic wrapping of cucumbers remains useful. The perceived greenness of cucumbers in the IMEA cultivar also differed under different light treatments in which the cucumbers grown in combination with intracanopy LED displayed the darkest color. These cucumbers also showed less of a decline in perceived greenness after storage than cucumber from the light treatments using only overhead lighting. This suggests a positive response to the intracanopy lighting producing more chlorophyll in the fruit's exocarp and thus enhancing the photosynthetic

rate of fruits. Both the IMEA and DeeRect cucumbers exhibited lower perceived greenness in response to storage and an increased yellow color, supported by the findings of Verheul *et. al.*, (2013) for both Spanish and Norwegian cucumbers after storage. The cucumbers from the DeeRect cultivar were on average greener than the IMEA cultivar and generally had a higher resilience to changes in the physical parameters measured than the IMEA cultivar as a function of different light treatment. The DeeRect cultivar did on the other hand show significance to all mentioned physical parameters between fresh and stored cucumbers as opposed to IMEA, though the standard deviation in the DeeRect cultivar was smaller than that of IMEA suggesting that stored cucumbers compared to fresh cucumbers from the IMEA cultivar displayed too large variance to demonstrate significance.

5.2 Results of chemical parameters

From the chemical parameters SSC, pH, TTA and the important relationship between them for taste perception, only DMC and SSC showed significantly different values with regards to light treatment for the cultivar IMEA, with the light treatment overhead HPS in combination with intracanopy LED again producing cucumber with higher concentrations SSC, probably as a result of a high PAR availability and the enhanced light spectrum quality from the intracanopy LED. The use of only overhead LED however resulted in cucumbers with higher SSC values in the IMEA cultivar compared to the use of only overhead HPS, despite irradiating a lower total PAR level (Table 3). Since a significant proportion of DMC consists of SSC it should be expected that DMC may follow suit and indeed the same light combination of overhead HPS in combination with LED produced cucumbers with the highest DMC for the IMEA cultivar, though these values were not significantly different from the other light treatments with the exemption of overhead HPS. It is unclear why overhead HPS alone produced cucumbers with significantly lower DMC for the IMEA cultivar, as Marcelis (1996) presented sink strength as dependent on growth temperature and the light treatment overhead HPS should provide higher leaf and fruit temperatures than the overhead LED treatments. The fact that the light treatment overhead LED showed the same significance in terms of high DMC as the light treatments with intracanopy LED is however promising for the future implementation of LEDs as an overhead light source. The increase in TTA/SSC for both the IMEA and DeeRect cultivars is due to a significant increase in TTA concentration for both cultivars after storage, whilst SSC concentrations remain constant with regards to storage. An increase in TTA content after storage in cucumbers is supported by Verheul *et. al.*, (2013), who found that the proportion of citric acid increases in cucumbers as a function of storage, whilst the proportion of malic acid decreases from 64% when fresh to 57% when stored for 4 weeks. This is noted to be due to an increase of citric acid rather than a decrease in malic acid, suggesting an overall increase in acid content.

Despite perceived greenness showing significant difference in terms of different light treatments for IMEA, no correlating significant difference was found for pigment concentration in either chlorophyll content or carotenoid content. Likewise, no such difference was found in the DeeRect cultivar. These conflicting results may be explained by the notable standard deviation for pigment measurements, weakening any significant findings in addition to the nature of the analysis. Since perceived greenness is based on the pigment content of the exocarp, whilst the pigment measurements were based on whole homogenized cucumbers, the pigments concentration in the homogenized samples were diluted compared to the pigments in the exocarp. This is supported by the fairly similar concentration patterns of measured pigments to perceived greenness in response to different light treatments. Some of the samples also exhibited absorbance values under 0.3, falling below the threshold for linear correlation between pigment concentration and absorbance (Lichtenthaler and Buschmann, 2001). Using cucumber peel, with a higher pigment content than whole fruit homogenate might mitigate these results. Another important explanatory factor might by the nature of the extraction solvent acetone which readily evaporates at room temperature. It is therefore possible that some samples in the wells evaporated more than other, concentrating some

samples over others. The chlorophyll content of both cultivars did significantly decrease as a response to storage however, supported by the perceived greenness score, though it is notable that the concentration of carotenoids increased in the DeeRect cultivar, but decreased in the IMEA cultivar as a response to storage.

Vitamin C content in fruits and vegetables is known correlate positively with increased exposure to red and blue light spectrals (Verkerke et. al., 2014; Hasan et. al., 2017). This relationship was confirmed in the IMEA cultivar cucumbers, in which the light combination overhead HPS in combination with intracanopy LED produced cucumbers with significantly higher vitamin C concentrations than the other light treatments. The same patterns were seen in the DeeRect cultivar, though standard deviations were too large to confirm such a significance. This does not explain why the light combination overhead LED in combination with intracanopy LED produced lower values of vitamin C than the combination with overhead HPS. A possible explanation is the increased temperature of both leaves and fruit of cucumbers cultivated under overhead HPS lamps due to the higher near infrared and infrared radiation emitted from these light sources, shown in Figure 5. This is supported by Zhen and Bugbee (2020), who demonstrated that far-red (701-750 nm) radiation elicits photosynthetic activity equivalent to that of traditionally defined PAR at levels up to 30% of total photon flux. This temperature increase might further upregulate photosynthesis and is reflected in higher SSC values for the specific light combination overhead HPS in combination with intracanopy LED on the IMEA cultivar. As with the physical measurements, the DeeRect cultivar again exhibited more favorable results across all tested chemical parameters in general, though no clear differences between the cultivars were found regarding improved quality after storage.

The ionic content as a response to different light treatments varied substantially between the IMEA and DeeRect cultivars, though most ionic species increased as a function of storage for both cultivars. This is an anticipated result, as subsequent water loss through transpiration during storage naturally increases the corresponding ionic concentration in fruits. It is therefore noteworthy to look at the exceptions from this

pattern, in particular NO_{3⁻}. The levels of NO_{3⁻} in both the IMEA and DeeRect cultivar did not increase significantly after storage and with regards to the IMEA cultivar. NO₃alongside NH₄⁺ are crucial ionic species for plants growth, key to amino acid and chlorophyll assembly in plants (Campbell et. al., 2017). Most photoautotrophic plants are unable to synthesize NO₃ (Hipkin *et. al.*, 2004) and must therefore acquire it externally. NO₃⁻ is then either stored in vacuoles or reduced by nitrate reductase (NR) to NO₂⁻ in the cytosol (Liu, et. al., 2014). It is known that NR is substrate inducible (Melzer et. al., 1989), hence an increase in NO₃⁻ would produce elevated NR levels and the NO₃⁻ content in the cell would be self-limiting, diminishing the effect of increased NO₃⁻ concentration due to water loss during storage. NR expression is also positively linked to increased light levels (Melzer et. al., 1989) and though significance could not be confirmed, the cucumbers in both light treatments including intracanopy lighting for the IMEA cultivar had lower NO₃⁻ levels than the light treatments utilizing only overhead lighting, supporting such an effect. It is interesting that the highest amounts of NO₃⁻ were found in the cucumbers subjected to the light treatment overhead HPS and in the IMEA cultivar significantly, suggesting a higher expression of NR in response to LED lighting, supporting increased photosynthetic efficacy of these light sources.

The DeeRect cultivar Na⁺ levels also did not show significant differences between fresh and stored cucumbers. It should be mentioned though that the Na⁺ results along with the of the NH₄⁺ concentration results were below the detection limit after blank subtraction for the IC-CD, creating negative concentrations. Coupled with a significant disparity in variation for both ionic species, the analytical results of Na⁺ and NH₄⁺ for both cultivars are questionable. Improved analytical techniques such as performing the IC-CD measurements for cations with the use of suppression might ameliorate these results. When reviewing the calibration points for the NH₄⁺ standards, only one calibration point was determined resulting in an insufficient calibration and resulting analysis of NH₄⁺. Why the concentration of Ca²⁺ was not significant between fresh and stored DeeRect cucumbers is unclear.

6. Conclusion

Light quality and quantity affected both physical and chemical parameters in the cucumbers cultivars analyzed in this thesis. The IMEA cultivar demonstrated overall greater variability than the DeeRect cultivar in both physical and chemical measurements, with important consumer preference parameters including length, weight and SSC increasing in response to the use of both LED overhead and intracanopy lighting as opposed to overhead HPS lighting. The use of LEDs also significantly increased the perceived greenness in the IMEA cultivar suggesting an upregulation of photosynthetic rate. This was indeed supported by a decrease in NO_{3} concentration in IMEA cucumber grown with intracanopy LEDs. The DeeRect cultivar grown under LED overhead lighting also had the highest perceived greenness. This indicates that the use of LED overhead lighting can produce darker colored cucumbers than traditional HPS overhead lighting, a desired consumer trait. The different light treatments used did not affect the TTA/SSC proportion significantly in either cultivar, which is a key factor for taste. This may also be reflected in the results from the taste test which also did find differences in taste preference for the IMEA cucumber cultivar. The effect of storage had more uniform patterns on both physical and chemical parameters for both cultivars. Light treatment did not affect the outcome of storage with the exception of perceived greenness for the IMEA cultivar and total carotenoid content for the DeeRect cultivar. It should be noted that the DeeRect cultivar had better overall values for the same parameter, indicating that the effect of cultivar choice is more significant for both physical and chemical parameters than light treatment. The findings support that the light treatment overhead HPS in combination with intracanopy LEDs seem to provide consistently favorable results, though many of the light treatments were marked by inconsistent results. More measurements on different cucumber cultivars would provide important insight towards confirming the role of light source on physical and biochemical parameters.

7. References

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