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Quality of Norwegian tomatoes: Effects of hybrid supplemental lighting systems on sugar content in tomato fruits grown in greenhouses

MASTER'S THESIS IN BIOLOGICAL CHEMISTRY

SONJA GINNARD

Abstract

The effects of supplemental lighting on greenhouse crops have been of recent interest, especially to those countries with seasons of limited natural light. This study aims to analyze the effects of both high-pressure sodium (HPS) top lighting with intra-canopy light-emitting diodes (LED), and to find a recommended treatment for efficient growth of high-quality tomatoes in Norway. This combination lighting system is predicted to have positive effects on the sugar contents of tomato fruit, especially on glucose and fructose concentrations, thus improving tomato quality. Ion chromatography (IC), gas chromatography-mass spectrometry (GC-MS), and absorption spectroscopy were used to first determine the most effective method to analyze sugar content in this study. IC was determined to be the best fit and was further utilized to analyze glucose and fructose concentrations across seven light treatments of various HPS top lights and LEDs. The concluded recommendation to Norwegian farmers from this study is a HPS top light with flux intensity of 263 W/m^2 in combination with 70 W/m^2 intra-canopy LEDs.

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1. Abbreviations

CD	Conductivity detector
DMC	Dry matter content
DW	Dry weight
FW	Fresh weight
GC	Gas chromatography
GC-MS	Gas chromatography-Mass spectrometry
HPLC	High performance liquid chromatography
HPS	High pressure sodium
IC	Ion chromatography
LED	Light-emitting diode
MEOX	Methoxyamine hydrochloride
MS	Mass spectrometry
NIBIO	Norwegian Institute of Bioeconomy Research
PAD	Pulsed amperometric detector
PPM	Parts per million (µg/mL)
VIS	Visible spectroscopy

2. Introduction

2.1. BioFresh project

The Norwegian Institute of Bioeconomy Research (NIBIO) is a government owned organization aimed at managing food and resource production in Norway (Nibio, 2019). They utilize researchers in various fields to carry out projects that can directly benefit Norwegian food and agriculture (Nibio, 2019). NIBIO's main goal is to foster the collection of knowledge within the fields of food and forestry for the good of businesses, society, and the nation (Nibio, 2019).

BioFresh is a four year long, NIBIO-sponsored project which focuses on environmentally sustainable improvements to Norway's greenhouse fruit and vegetable production (Nibio, 2019). The Norwegian climate is not always conducive to growing quality crops, so the use of greenhouses has become utilized as means to fill the gap between consumer demand and product availability. Greenhouse producers in Norway have done well thus far with attaining high yield crops without sacrificing quality (Nibio, 2019). With current demand, it has been speculated that food production will need to increase by roughly 50% in the next 40 years (Murchie et al., 2009), an effort which greenhouse optimization can be of great use. However, at the moment greenhouse production uses large amounts of energy and comes with the significant costs of construction and maintenance (Nibio, 2019). Biofresh's goal is to research and utilize new technologies to lower greenhouses' energy use while producing high quality, high yield products and reducing their climate impact (Nibio, 2019). Optimization of energy usage and recycled organic nutrients is followed by quality testing to determine the future potential of greenhouse production in Norway (Nibio, 2019).

2.2. Supplemental light in greenhouse tomato production

Applying a second light source, in addition to natural light, to plants grown in greenhouses can make up for a lack of sunlight needed for proper plant and fruit development. Moderate regions of Norway, for example, can have only four hours of daylight in the middle of winter while the more northern regions do not see the sun for months. In an effort to reduce importation, supplemental lighting can be used to successfully grow domestic produce regardless of the available daylight.

There have been many studies to optimize supplemental lighting conditions for various plants in the past decades (Olle and Viršile, 2013; Pinho et al., 2012). What began with adding one light source to natural light, evolved into the relatively new practice of combining and optimizing multiple light sources. HPS lamps and LEDs, of various wavelengths, have been compared and combined to increase productivity and quality. HPS lamps have long been the standard for supplemental lighting in greenhouses (Olle and Viršile, 2013), while LEDs are relatively new in crafting hybrid greenhouse lighting systems (Pinho et al., 2012). In addition to LEDs becoming more widely recognized in greenhouse production, they are becoming more energy efficient with time and are expected to vastly surpass the HPS lamps' effectiveness by 2020 (Olle and Viršile, 2013; Pinho et al., 2012).

2.3. Tomato plant metabolism

Studying the metabolism of developing tomato fruits is an important step in understanding and increasing quality for consumers. Tomato plants in particular experience major metabolic

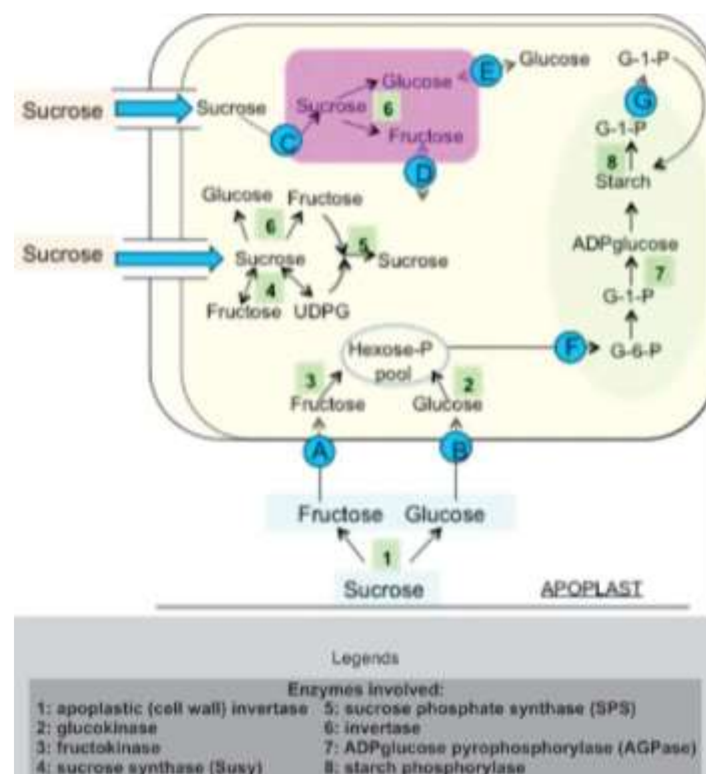

Figure 2.1. Metabolism pathways involving the enzymatic breakdown of sucrose into glucose and fructose by invertase (6) and sucrose synthase (4). Sucrose enters the cell through either the symplast (left) or indirectly through the apoplast (bottom). Starch is synthesized in the plastid (green) by starch phosphorylase (8). The main sugars sucrose, glucose, and fructose are stored in the vacuole (violet) (Beckles et al., 2012).

changes during the course of their development (Carrari, 2006). There are five phases of fruit development: antithesis, fertilization, cell division, cell expansion, and ripening. The ripening phase invites much focus, as this is when fruit quality parameters, such as soluble solid content (SSC), are determined (Beckles et al., 2012). Ripening is initiated by ethylene and hosts a rapid import and accumulation of sugars alongside starch degradation (Figure 2.1.). Sucrose is imported into the cell through the symplast or it is metabolized into glucose and fructose which are imported via the apoplast, and all three major sugars are stored in the vacuole (Figure 2.1) Starch can be broken down into individual glucose units, further contributing to sugar content. Additionally during ripening, chlorophyll degrades while lycopene and carotenoids are synthesized, which results in a gradual color change from green to red (Beckles et al., 2012).

2.4. Tomato quality

Tomato plants and fruits have been of interest to producers, consumers, and researchers for more than 50 years (Davies et al., 1981). There has been a wide focus on producing high yields of tomatoes that fit size and shape requirements for ease of storage and handling post-harvest (Davies et al., 1981). Since these plants thrive in warmer, humid climates with regular day/night cycles, greenhouses have been employed to have constant control over growth conditions to produce quality crops in any climate (Olle and Viršile, 2013). Due to this control offered by greenhouses, Norway has been able to increase its tomato production, therefore decreasing the amount required to be imported. Greenhouses solve the productivity issue, but the natural next step is utilizing research findings to produce not only high yield, but high-quality tomatoes for the consumers of Norway.

The balance between sugars and acids in a tomato largely dictate its flavor quality and a sweeter tomato is usually perceived as a better tasting tomato. It has been found that reducing sugars, those containing an aldehyde or ketone group giving it the potential to act as a reducing agent, make up roughly 50% of the dry matter content (DMC) of a tomato. Of these are predominantly fructose and glucose, with sucrose levels rarely exceeding 0.1% of fresh weight. There has been extensive research carried out to identify the main constituents in tomato fruits and how they can be manipulated using environmental factors (Davies et al., 1981).

While it has been found that supplemental light can almost double tomato plant growth rate and decrease the time needed to first flower (Canham, 1974), it is of interest to scientists what effect additional light can have on important flavor constituents, such as glucose and fructose. Crop yield and total soluble sugar concentration in fruits have been found to have an inverse correlation in tomato plants (Prudent et al., 2009), most likely due to an issue of dilution in fruits. Light is considered the most important environmental factor with regards to sugar concentrations (Davies et al., 1981). However, too much light or too direct light can reduce fruit quality by way of sun damage to the skin and impaired lycopene development (Dorais et al., 2010; McCollum, 1954).

2.5. Ion chromatography

Ion chromatography (IC), sometimes referred to as ion-exchange chromatography (IEC), is a type of high performance liquid chromatography (HPLC) which separates a mixture of ions by elution (Fritz, 2004). An IC system contains a stationary and a mobile phase, where the mobile phase is an ionic solution kept at a constant flow rate and pressure by a specialized pump (Fritz, 2004). Depending on the need, an IC column can be filled with specially manufactured cation-exchange or anion-exchange material to serve as the stationary phase (Fritz, 2004). These are used to separate cations and anions, respectively. The mobile phase is first washed through the column to bind all ions in the stationary phase. The sample is then applied, and analyte ions replace eluent ions and bind to the stationary phase. Eluent is flushed through the system and analyte ions elute in the order of their ionic strength and how strongly they were bound to the stationary phase ions. Ions travel through the system to a detector and a chromatogram is produced showing separated compounds (Fritz, 2004).

Many components of an IC system can vary based on the type of analyte and sample. The column, as previously mentioned, can be selected to analyze either cations or anions. The detector can be selected based on the properties of the sample to be analyzed. Many detectors have been used with IC, such as conductivity detectors (CD), pulsed amperometric detectors (PAD), refractive index (RI), and spectroscopy (Steinbach and Wille, 2010). Today, IC coupled with PAD is the most widely used method for detecting carbohydrates in food samples (Pereira da Costa and Conte-Junior, 2015). At very high pH ($pH \geq 11$), the hydroxyl groups in sugars are ionized and they become anionic, making them a great candidate for

anionic chromatography. In addition to this, very little sample preparation is required for analyzing food samples on IC compared to other analysis methods (Steinbach and Wille, 2010). While CD has long been prevalent as a detection method in IC, in recent years PAD, equipped with a gold working electrode, has been found to be ideal for carbohydrate analysis due to its higher selectivity in alkaline solutions (Fritz, 2004; Pereira da Costa and Conte-Junior, 2015).

2.6. Gas chromatography-mass spectrometry

Gas chromatography (GC) is another type of chromatography that utilizes a gas mobile phase with a solid stationary phase, where separation occurs by partitioning between the two. Its column is much longer and thinner than IC, and it requires a specific sample type. Samples to be analyzed with GC should be low in molecular weight, and they must be volatile or be capable of being made volatile by chemical derivatization. This obviously adds a more complicated, though not impossible, sample preparation for analytes, such as sugars in tomato flesh, that are not volatile (Hill and Roessner, 2013).

GC coupled with mass spectrometry (GC-MS) is widely known for its robustness in quantification, its sensitivity, and for its ability to achieve very powerful separation and identification of compounds. It can be used for a detailed look into plant metabolism. Electron ionization MS is the go-to detector for metabolite analysis, as it is older and has been well studied and well developed. The role of a MS in this instrumentation is to fragment compounds into smaller ions and separate these based on their mass-to-charge ratio (*m/z*). This results in a mass spectrum which, along with retention time (RT), can be used to identify a compound by library comparison. The generally used procedure for analyzing plant metabolites by GC-MS is relatively straightforward. It involves homogenization and extraction of sample, derivatization, separation on GC column, and fragmentation by MS. Derivatization is the laborious and time-intensive aspect of this analysis method, and one of its drawbacks. A typical derivatization for polar metabolites is a trimethylsilylation (Figure 2.2) with methoxyamine hydrochloride (MeOX) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), where MSTFA introduces a trimethylsilyl group to replace the hydrogen in the hydroxyl which makes the molecule more volatile (Hill and Roessner, 2013).

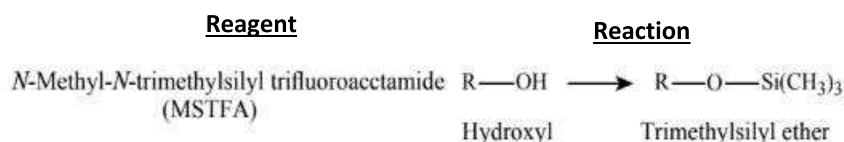

Figure 2.2. Reaction scheme of silylation derivatization by MSTFA for GC sample preparation. MSTFA substitutes the hydrogen in the hydroxyl group with a trimethylsilyl group, forming a trimethylsiloxy, thus enhancing the molecule's volatility (Hill and Roessner, 2013).

Data is typically log-transformed to minimize outlier effects and then run through a powerful statistics analysis, such as principle component analysis (PCA) or analysis of variance (ANOVA). This data can be very informative and has been shown to describe similarities and differences between plants grown in different environmental conditions (Hill and Roessner, 2013).

2.7. Absorption spectroscopy

Spectrophotometry is a commonly used umbrella term to describe the absorbing, transmitting, or reflecting of a sample's radiant energy (Porcu and Leder, 2018). Absorption spectroscopy falls under this umbrella, and is used to measure the absorbance of molecules within the ultraviolet and visible ranges, 350 -750 nm (Porcu and Leder, 2018). The basic principle behind this type of spectroscopy involves a liquid sample being exposed to a specific wavelength of light. The extent to which the molecules absorb the light can be directly used to calculate concentration of the analyte (Porcu and Leder, 2018). The calculations are performed based on the Beer-Lambert law, $A = \varepsilon bc$, where A is absorbance, ε = molar absorptivity, b = pathlength, and c = analyte concentration.

Visible absorption spectroscopy is sometimes used in food quality research, often as a method of screening prior to in-depth quantitative analysis (Porcu and Leder, 2018). It has mostly been used to analyze large molecules, such as proteins, in food samples, but seems to be less prevalent in food analysis than other available techniques (Roberts et al., 2018). However, absorption spectroscopy is known to be a very quick, inexpensive, and relatively simple way of detecting chromophores in samples (Roberts et al., 2018), so it can be a good option with the appropriate sample.

2.8. Objectives

The main objective of these experiments is to determine whether supplemental light affects sugar concentrations in tomato fruits. Glucose and fructose were the main focus, as these have been found to be the most prominent sugars making up about half of the dry matter content of a tomato fruit. The hypothesis was that supplemental light, in the form of HPS lamps and/or LEDs, will increase sugar concentrations and therefore produce a higher quality fruit (Dzakovich et al., 2015; Haque et al., 2015; Kowalczyk et al., 2012; Olle and Viršile, 2013).

A secondary objective is to compare three methods of sugar analysis: IC, GC-MS, and visible spectroscopy. A select group of samples were analyzed for glucose concentrations using all three methods, and the best method was used to continue with all other sample analyses for glucose and fructose concentrations. The hypothesis was that IC would give the best and most reliable data for these specific analyses (Pereira da Costa and Conte-Junior, 2015).

3. Materials and Methods

3.1. Materials

The chemicals used in all experiments in this study, with their corresponding details are listed in Table 3.1.

Table 3.1. Details of chemical materials used in all experiments.

Name	CAS	Supplier	Product No.	Country
Glucose analytical standard	50-99-7	Sigma-Aldrich	47249	USA
Fructose analytical standard	57-48-7	Sigma-Aldrich	F0127	USA
Sucrose analytical standard	57-50-1	Supelco	47289 (1)	USA
α-Lactose analytical standard	5989-81-1	Sigma-Aldrich	47287-U	USA
Lactulose analytical standard	4618-18-2	Sigma-Aldrich	61360	USA
Inositol analytical standard	87-89-8	Sigma-Aldrich	PHR1351	USA
Ribitol analytical standard	488-81-3	Supelco	47266	USA
Threitol analytical standard	2418-52-2	Sigma-Aldrich	377619	USA

Sodium Hydroxide	1310-73-2	Merck	105587	Germany
Starch Colorimetric/Fluorometric Assay Kit	n/a	Biovision	K647	USA

The instrumentation used for all analyses in this study, with their corresponding details, are listed in Table 3.2.

Table 3.2. Details of instrumentation used in all experiments.

Instrument model	Software	Manufacturer	Country
883 Basic IC Plus	MagIC Net 3.2	Metrohm	Switzerland
945 Professional Detector Vario	MagIC Net 3.2	Metrohm	Switzerland
863 Compact Autosampler	MagIC Net 3.2	Metrohm	Switzerland
CH-150 Column Heater & Controller	n/a	ESA	USA
Multiskan GO	SkanIt Software 5.0	Thermo Scientific	USA
794 Basic Titrino	n/a	Metrohm	Switzerland
Palette Digital Refractometer	n/a	Atago	Japan
Durofel Firmness Tester	n/a	Agro Technologies	France
Jouan B4i Centrifuge	n/a	Thermo Scientific	USA
Agilent 6890 Gas Chromatograph	MassHunter B.07.00	Agilent Technologies	
HP-5ms (30 m, 0.25 mm, 0.25 µm) capillary GC column	n/a	Agilent Technologies	USA
5975 Inert Mass Selective Detector	MassHunter B.07.00	Agilent Technologies	USA
BK-FD10S Freeze Dryer	n/a	BIOBASE	China
Repromer RCX-30 (250 x 4 mm ID) HPLC column	MagIC Net 3.2	Dr. Maisch	Germany

3.2. Growth conditions

Solanum lycopersicum 'Dometica' (Dometica) tomato seeds were sown on August 13, 2018 and were grown hydroponically in a climate-controlled greenhouse at NIBIO Særheim in southwest Norway. The plants were exposed to seven different light treatments, a combination of high-pressure sodium lights (HPS; Gavita Nordic, Norway) and light-emitting diodes (LED) while all other factors remained constant (Table 3.3).

Table 3.3. Seven different light treatments and the corresponding parameters of their growth compartments and applied light. 750 W HPS refers to a GAN 750 - W 150 DE - 750W light fixture with a Philips GP Plus 750 lamp; 600 W HPS refers to a GAN 600 - HR96 SE - 600W light fixture with a Philips GP Plus 600 lamp.

Treatment	Compart-ment	Measured temperature (°C)	Measured humidity (%)	HPS irradiance (W/m²)	HPS type (W)	LED irradiance (W/m²)
T176 K T176 LED 70 T176 LED 140	20	21.3	68	176	750	0 (control) 70 140
T263 K T263 LED70	21	22.3	75	263	600	0 (control) 70
T300 K T300 LED70	22	22.4	74	300	600/750 mix	0 (control) 70

Each LED fixture contains 20% blue diodes (450 nm) and 80% red diodes (660 nm) and has an intensity of 262 W/m^2 at a 10 cm distance.

Figure 3.1. Tomatoes growing in NIBIO greenhouse, with intra-canopy LED strips.

3.3. Laboratory analyses

3.3.1. Basic quality measurements

A total of 378 tomatoes, of color 8, were harvested for basic quality measurements, including firmness, SSC, and total titratable acidity (TTA). The tomatoes were harvested in three blocks on various dates between December 2018 and January 2019 (Table 8.1). The samples from each light treatment were measured in triplicate, with each replicate consisting of 6 tomatoes of equal size representation. Size was measured by eye, and color was measured by comparison with a designated color chart (Figure 3.2).

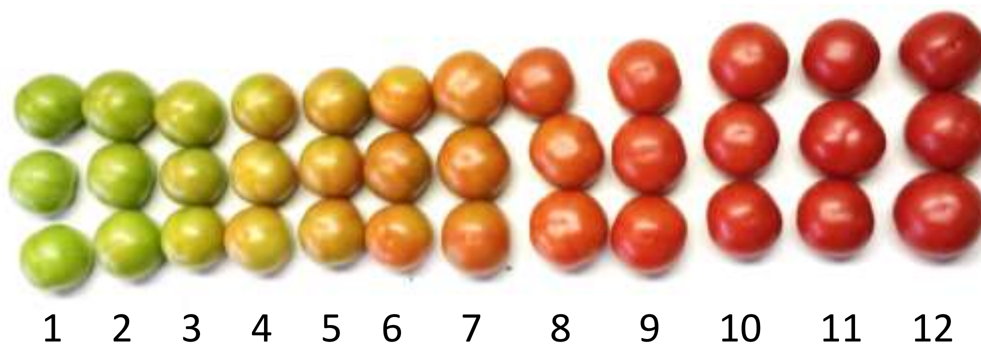

Figure 3.2. A visual representation of each of the 12 color grades during tomato fruit development, where color 8 is the ideal harvest color for sellers and the color with which the following experimentation was done.

<u>Firmness</u>

Firmness was measured using a Durofel firmness tester (Agro Technologies, France). Each tomato per replicate was probed three times, and all 18 values were averaged to give one firmness value for the replicate.

<u>Homogenization</u>

One quarter, cut from top to bottom, of each of the six tomatoes was taken for homogenization of each replicate. Homogenization was carried out by Multiquick 5 immersion blender (Braun, USA).

<u>Titration</u>

Titration was carried out according to the methods by Mitcham et al. (1996). Approximately 5 g of homogenate were stirred into a beaker with 50 mL distilled water. 100 mM NaOH was

used to titrate the sample to pH 8.2, using the end point setting on the Titrino instrument (Metrohm, Switzerland).

Solid Soluble Content

SSC determination was carried out according to the methods by Mitcham et al. (1996). Refractive index was measured using a Palette digital refractometer (Atago, Japan). Values are given in °Brix, or SSC percent. Distilled water was used to blank the refractometer.

Storage

Aliquots of each sample were added to Eppendorf tubes, frozen in liquid nitrogen, and stored in -80°C until further chemical analyses.

Figure 3.3. Tomato homogenate divided into aliquots for storage.

3.3.2. Ion Chromatography

All samples and standards analyzed with ion chromatography (IC) used the following parameters: 1 mL/min flow rate through a HPLC column (250 x 4 mm) kept at 25°C, isocratic elution with 100 mM NaOH, and a pulsed amperometric detector (PAD) kept at 35°C. All dilutions were made with ultrapure distilled water filtered using a 0.45 µm nylon filter under vacuum. All samples were analyzed in a random order. Distilled water was used as a blank and was run through the instrument every three samples.

Sample Preparation

Soluble sugar extraction procedures for all experiments were adapted from Smith and Zeeman (2006). Approximately 0.5 g tomato homogenate were vortexed together with 5 mL ethanol (80% v/v). The samples were placed in a heating block at 100°C for 3 min and

centrifuged at 2880 g (Thermo Scientific, USA). The supernatant was transferred to a new tube and the extraction procedure was repeated twice more. The final combined supernatant was diluted 6 times with distilled water and filtered through a 0.45 µm filter syringe (Branchia, UK).

Standard Curve

Seven standards containing 1, 2, 10, 50, 100, 150, and 200 ppm each of glucose, fructose, sucrose, and inositol in distilled water were analyzed using IC. This standard curve was used for quantification of these four compounds in the samples.

Standard Curve – Method Comparison

Seven standards containing 0.5, 1, 5, 25, 100, 250, and 500 ppm glucose in distilled water were prepared and each diluted 11 times, to be directly compared with results from spectroscopy. They were analyzed using IC.

3.3.3. Gas chromatography-mass spectrometry

All samples and standards were analyzed by an Agilent 6890 gas chromatograph with an Agilent 5975 inert mass selective detector equipped with an Agilent HP-5ms 30 m nonpolar capillary column, with 0.25 mm ID and 0.25 µm film.

Sample order was randomized and a pulsed splitless injection of 1 µL of sample at 230 °C was performed by autosampler. A blank of EtOAc was run every 3 samples. The pulse pressure was 200 kPa with a 2 min pulse time. Purge time was 1.9 min at a rate of 30 mL/min. The inlet pressure after injection was 57 kPa.

Helium was used as the carrier gas and flowed at a rate of 1 mL/min. Each sample ran through a 56 min program, which consisted of an initial injection temperature of 60 °C, which increased by 5 °C/min until it reached 325 °C, where the temperature was held for the final 2 min.

The MS was tuned using perfluorotributylamine as per the manufacturer's instruction. Electron ionization was employed at 70 eV. Mass spectra were recorded at 5.5 scans/s with an *m/z* scanning range of 50-550 amu, and spectra recording began at 5 min.

Compounds were identified by comparison with a previously created library which includes mass spectra and retention times. Ribitol was used as an internal standard, and all peak areas were normalized to the corresponding ribitol peak area.

Sample Preparation

100 µL of the same ethanol extract from T300 K and T300 LED70 used for IC were added to GC vials along with 5 µL aqueous ribitol solution with initial concentration of 5.63 mg/mL. Each sample was prepared in triplicate. Vials were frozen at -80°C for 30 min before being placed in the freeze dryer overnight for solvent removal.

Standard Curve

Eight standards containing 0.5 ppm sucrose, inositol, lactose, lactulose, and threitol; 1, 5, 25, and 100 ppm glucose, fructose, sucrose, inositol, lactose, lactulose, and threitol; and 250 and 500 ppm glucose and fructose in distilled water were prepared. 50 µL of standard mix was added to a GC vial with 5 µL aqueous ribitol solution with initial concentration of 5.63 mg/mL. Vials were frozen at -80°C for 30 min before being placed in the freeze dryer overnight.

Derivatization

A 20 mg/mL solution of MEOX in pyridine was prepared in a new GC vial. 40 µL of MEOX solution were added into each vial containing sample or standard. Vials were incubated for 90 min at 37°C. 1 mL *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) was added to a new vial with 10 µL alkanes (C_8-C_{40}). 80 µL of MSTFA solution were added into each vial containing sample or standard. Vials were incubated for 30 min at 37°C. Samples were analyzed using GC with conditions previously mentioned.

3.3.4. Visible spectroscopy

Cell Biolabs starch assay kit was used for spectroscopic analyses of glucose according to its given protocol, with modifications to only quantify the concentrations of soluble glucose.

Sample Preparation and Standard Curve

2 µL of the same ethanol extract from T300 K and T300 LED70 used for IC were added to wells of a microtiter plate. The samples were analyzed in triplicate with one control well per sample well. Seven glucose standards were prepared in concentrations of 0.5, 1, 5, 25, 100, 250, and

500 ppm in distilled water. 5 µL of each standard were added to its own well, and each standard series was analyzed in triplicate. Three wells were filled with 5 µL distilled water to serve as blank readings.

A Development Mix was prepared using 46 µL Development Buffer, 2 µL Development Enzyme, and 2 µL OxiRed Probe per 50 µL. 50 µL of Development Mix were added to every well containing sample, standard, or blank and the plate was incubated for exactly 30 min at 37°C in light-free conditions. Absorbance was measured at 570 nm at 37°C.

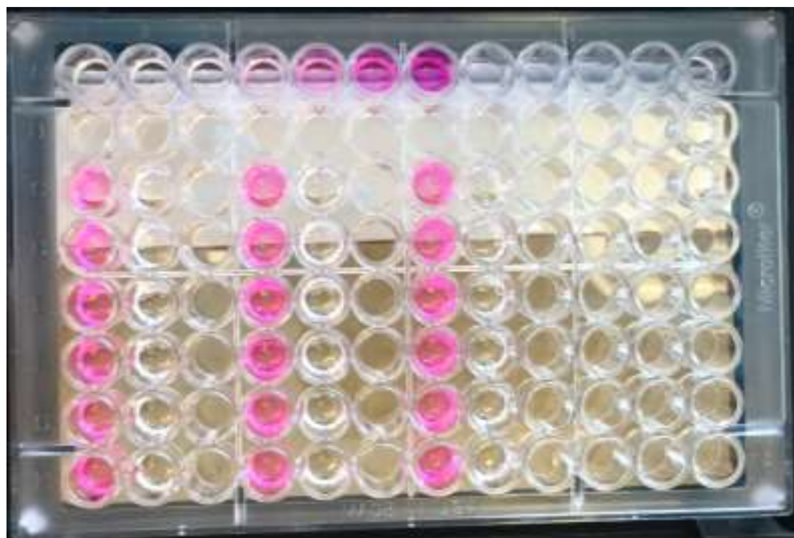

Figure 3.4. Samples and standards immediately after the final incubation with OxiRed Probe and before absorbance measurements were taken.

3.4. Statistics and data processing

Statistical analysis was carried out using SigmaPlot 14.0 and Microsoft Excel. Analysis of variance (ANOVA) was used to compare SSC across different light treatments and harvests, and to compare glucose and fructose concentrations across different analysis methods. Student's t-test was used to compare means of analyte responses from GC-MS analysis. A significance level $\alpha = 0.05$ was used for all statistical tests.

4. Results

4.1. Basic quality measurements

The following basic quality measurements (Figure 4.1) were performed as an initial step in evaluating tomato quality (Chapter 2.4, Chapter 3.3.1). SSC data from basic quality

measurements were used to decide which samples were to be further analyzed because SSC in tomato fruit is positively correlated with total sugar concentration (Dorais et al., 2010). A significance level α = 0.05 was used for all statistical tests.

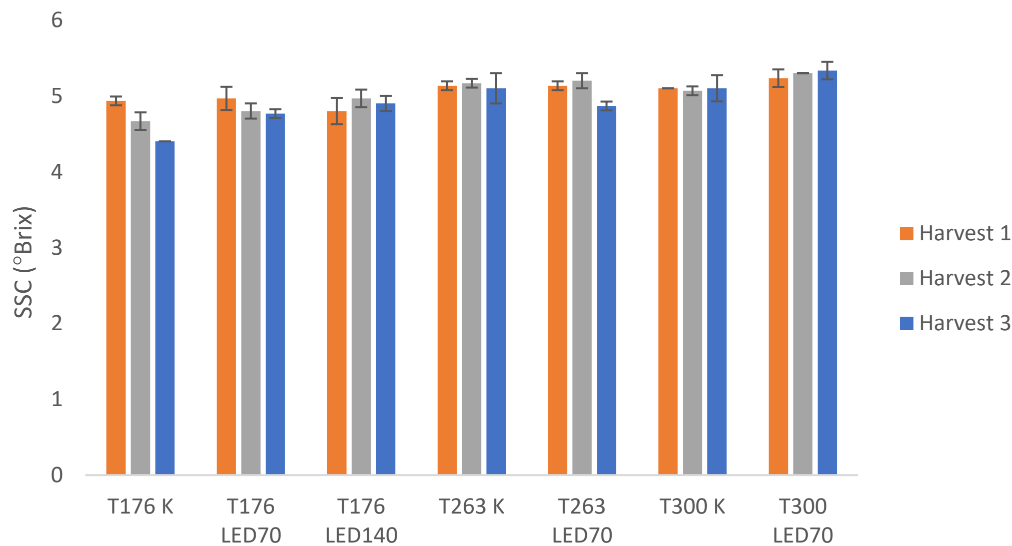

Figure 4.1. SSC, given in °Brix, for three replicates of each of the seven light treatments from the third harvest, with error bars showing standard deviation (n=3). Complete values from all three harvests can be found in the Appendix.

A three-way ANOVA was performed to determine the effects of top light, LED, and harvest number on Brix value. This test excluded LED140 treatments so that interactions could be analyzed. The test shows a statistically significant interaction between the three independent variables on SSC, $p = 0.013$, so main effects cannot be properly determined. This was followed by a Holm-Sidak test to determine interaction effects. The top light and LED interaction effect is not significant in harvests 1 or 2, $p = 0.486$, $p = 0.231$, respectively. However, the top light and LED interaction does have a significant effect on the Brix values in harvest 3 samples, $p = <0.001$. This test also shows significant differences between all top light treatments (T300 vs. T176 $p = <0.001$; T263 vs. T176 $p = <0.001$; T300 vs. T263 $p = 0.011$) and between LED70 and control, $p = <0.001$.

A two-way ANOVA was performed to determine the effects of LED and harvest number on SSC. This test included T176 treatments from all three harvests. The interaction effect of LED and harvest on Brix is significant. Further Holm-Sidak tests show that the most differences

among LED treatments occur within harvest 3 (LED140 vs. control *p* = <0.001; LED70 vs. control *p* = 0.001; LED140 vs. LED70 *p* = 0.151).

The results of these two ANOVA tests suggested that harvest 3 had the best potential for showing differences in sugar concentrations between treatments. Further analyses were performed on samples from harvest 3 only.

4.2. IC analysis

IC was used to quantify glucose and fructose concentrations in samples from all seven light treatments from harvest 3 (Chapter 3.3.2). After being compared with spectroscopy and GC-MS, this method showed to give the best results under the conditions used in this study and was therefore used as an analysis method for all treatments.

Figure 4.2 shows the calibration curves made from IC that was used to quantify glucose and fructose concentrations in all samples, using peak height for calibration.

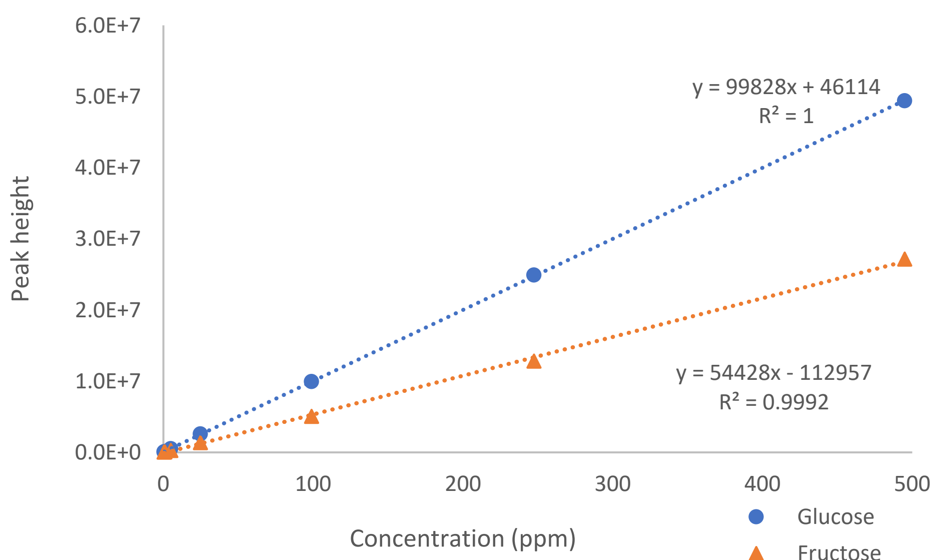

Figure 4.2. Calibration curve from IC for both glucose (blue; y = 99828x + 46114; R^2 = 1) and fructose (orange; y = 54428x - 112957; R^2 = 0.9992).

Preliminary two-way ANOVAs were carried out to determine whether data normalized to fresh weight or dry weight should be used. These analyses analyzed the effects of top light

and LED on glucose in terms of percent fresh weight and percent dry weight. Data normalized to dry weight of samples showed no significant interaction effect between top light and LED ($p = 0.496$), and no significant main effects of top light ($p = 0.453$) nor LED ($p = 0.329$). On the contrary, the data normalized to fresh weight of the samples showed a significant interaction effect between top light and LED. For this reason, data normalized to fresh weight was the main consideration for the rest of the study.

Figure 4.3 shows the calculated glucose and fructose concentrations for each sample according to IC, in terms of fresh weight.

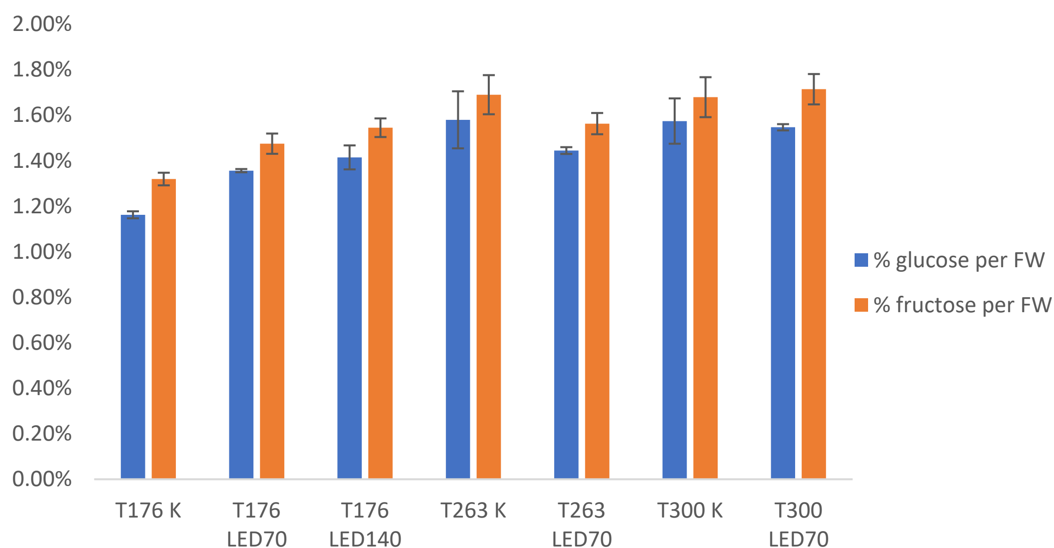

Figure 4.3. Values for calculated percentages of glucose and fructose per fresh weight tomato samples from seven treatments, error bars represent standard deviation (n=3).

A two-way ANOVA was performed to analyze the effects of top light and LED on the amount of glucose detected by IC, expressed as percentage of fresh weight, in harvest 3 samples. This analysis excluded LED140 treatments so that interactions could be analyzed. The test shows a significant interaction effect from top light and LED ($p = 0.003$), and that the effect of top light depends on the level of LED present. Further Holm-Sidak tests show a significant difference between control and LED70 within T176 ($p = 0.004$) and T263 ($p = 0.030$), but not within T300 ($p = 0.630$). Additionally, there is no difference between T300 and T263 (p =

0.245) across all the samples, and no difference between T300 and T263 ($p = 0.904$) within control samples.

A two-way ANOVA of the same nature was performed to analyze the effects of top light and LED on the amount of fructose, expressed as percentage of fresh weight, in harvest 3 samples. LED140 treatments were also excluded from this analysis so that interaction effects could be analyzed. This analysis shows a significant interaction between top light and LED ($p = 0.009$). Similar to the glucose ANOVA results previously mentioned, Holm-Sidak tests in this analysis also show significant differences between control and LED within T176 ($p = 0.013$) and T263 ($p = 0.032$), but not within T300 ($p = 0.497$).

A one-way ANOVA was performed to determine the effect of LED on glucose amounts detected by IC, expressed as percentage of fresh weight, in harvest 3 samples. Only T176 treatments were used in this test. The analysis shows a statistically significant difference between levels ($p = <0.001$). Further pairwise comparisons using the Holm-Sidak method show only significant differences between LED140 vs. control ($p = <0.001$) and LED70 vs. control ($p = <0.001$), but no significant difference between LED140 vs. LED70 ($p = 0.060$). The rest of the pairwise comparisons show no significant differences ($p \geq 0.05$).

A similar one-way ANOVA was carried out to determine the effect of LED on fructose concentrations detected by IC, expressed at percentage of fresh weight, in harvest 3 samples. Again, only T176 treatments were used in this analysis. There is a significant difference between levels ($p = <0.001$), but when further analyzed with the Holm-Sidak method, there is no significant difference between LED140 and LED70 ($p = 0.060$), but a difference can be seen between control and LED70 ($p = <0.001$).

Generally, the results showed significant differences between T176 and T263, but not between T263 and T300. Significant differences were also observed between control and LED70, but not between LED70 and LED140.

A full table of ANOVA results can be found in the Appendix.

4.3. GC-MS analysis

GC-MS analysis was used in part as a comparison of methods, and in part as a tool to semi-quantitatively determine differences between light treatments by use of internal standard

(Chapter 3.3.3). After each peak area was normalized to ribitol peak area and dry weight of the sample, a Student's t-test was performed for each of the seven analytes measured. The results of the t-tests (Table 4.1) were used to determine differences between two light treatments of harvest 3: T300 K and T300 LED70.

Table 4.1. Analytes measured using GC-MS and their corresponding statistics obtained from two-tailed Student's t-tests performed on the ribitol-normalized peak areas. Only T300 K and T300 LED70 from harvest 3 were used. SD = standard deviation (n=3).

Analyte	Treatment	Mean	SD	Difference of Means	*p*-value
Glucose	T300K	3.44E-02	1.63E-02	-1.02E-02	0.207
	T300 LED70	4.46E-02	1.65E-02		
Fructose	T300K	9.34E-04	5.16E-04	-3.99E-04	0.151
	T300 LED70	1.33E-03	6.05E-04		
Inositol	T300K	1.26E-05	4.13E-06	-3.01E-06	0.229
	T300 LED70	1.56E-05	5.93E-06		
Sucrose	T300K	-1.12E-08	1.21E-08	-1.10E-09	0.833
	T300 LED70	-1.01E-08	9.55E-09		
Lactose	T300K	1.72E-06	1.56E-06	-1.12E-06	0.149
	T300 LED70	2.83E-06	1.56E-06		
Lactulose	T300K	5.46E-09	9.82E-09	-1.56E-08	0.090
	T300 LED70	2.10E-07	2.40E-08		
Threitol	T300K	1.03E-04	2.08E-04	-1.48E-05	0.892
	T300 LED70	1.18E-04	2.44E-04		

4.4. Spectroscopic analysis

Spectroscopic analysis was performed on two treatments from harvest 3, T300 K and T300 LED70, to colorimetrically quantify glucose concentrations and further be compared to other methods studied (Chapter 3.3.4).

The calibration curve shown in Figure 4.4 was used to quantify all samples measured with visible spectroscopy.

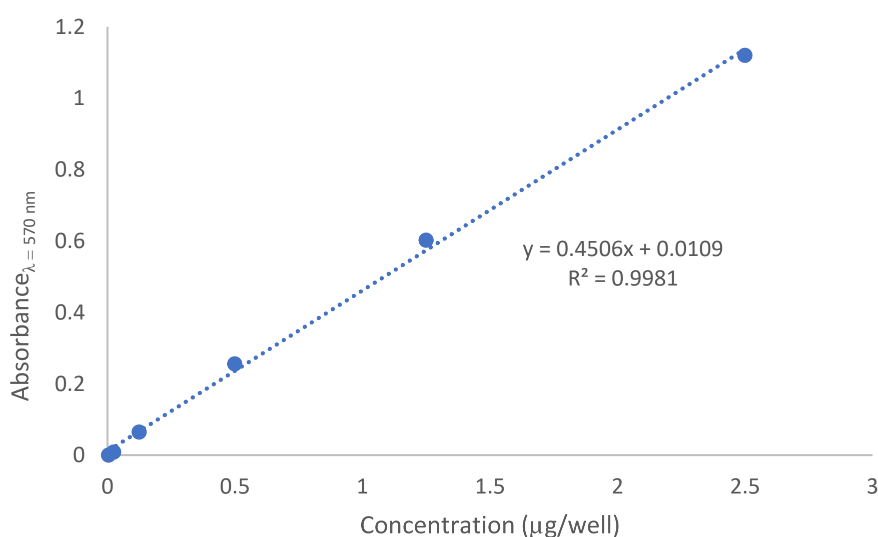

Figure 4.4. Spectroscopy calibration curve made from absorbance readings (λ = 570 nm) of glucose standards on visible spectroscopy (y = 0.4506x + 0.0109; R^2 = 0.9918).

Table 4.2 shows glucose percentages of dry and fresh weight (FW) for six tomato samples from two treatments. The values were calculated based on the calibration curve (Figure 4.4).

Table 4.2. Values for percentage of glucose per fresh weight for tomato samples from two treatments (n=3).

Treatment	% glucose of FW Mean ± SD
T300K	1.40% ± 0.08%
T300 LED70	1.44% ± 0.10%

A Student's t-test was performed to analyze the data given by spectroscopy (Table 4.2). At a 95% confidence level, the data do not show any significant differences between treatments T300K and T300 LED70 in harvest 3 (p = 0.637).

4.5. Method comparison

To visualize the comparison between spectroscopy and IC, absorbance was plotted against IC peak height (Figure 4.5). The instrument responses show a linear correlation (R^2 = 0.9985) in the glucose concentration range of 0.5 to 500 ppm.

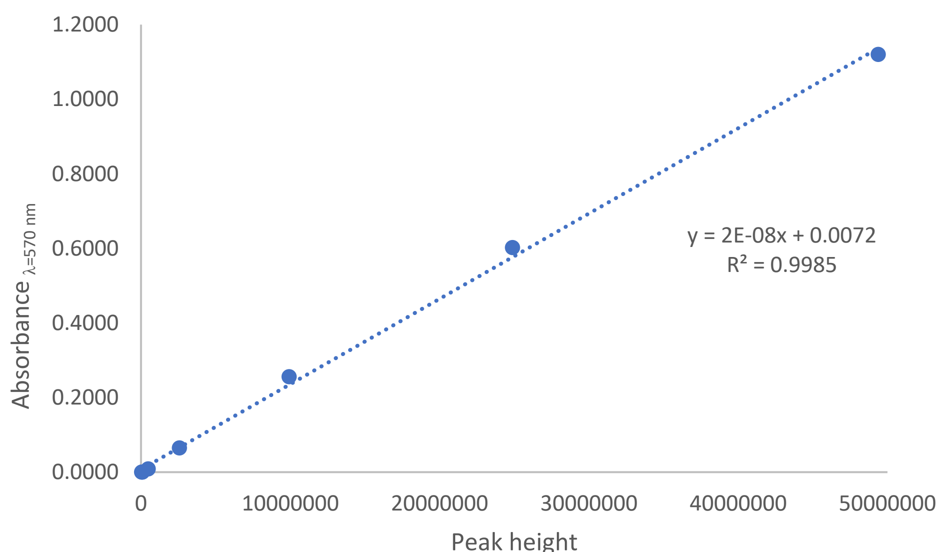

Figure 4.5. Glucose absorbance (λ=570 nm) values from spectroscopy readings versus glucose peak height from IC chromatograms (y = 2E-08x + 0.0072; R^2 = 0.9985).

Additionally, each calibration curve from both IC and spectroscopy were plotted on the same graph to visually determine differences (Figure 4.6).

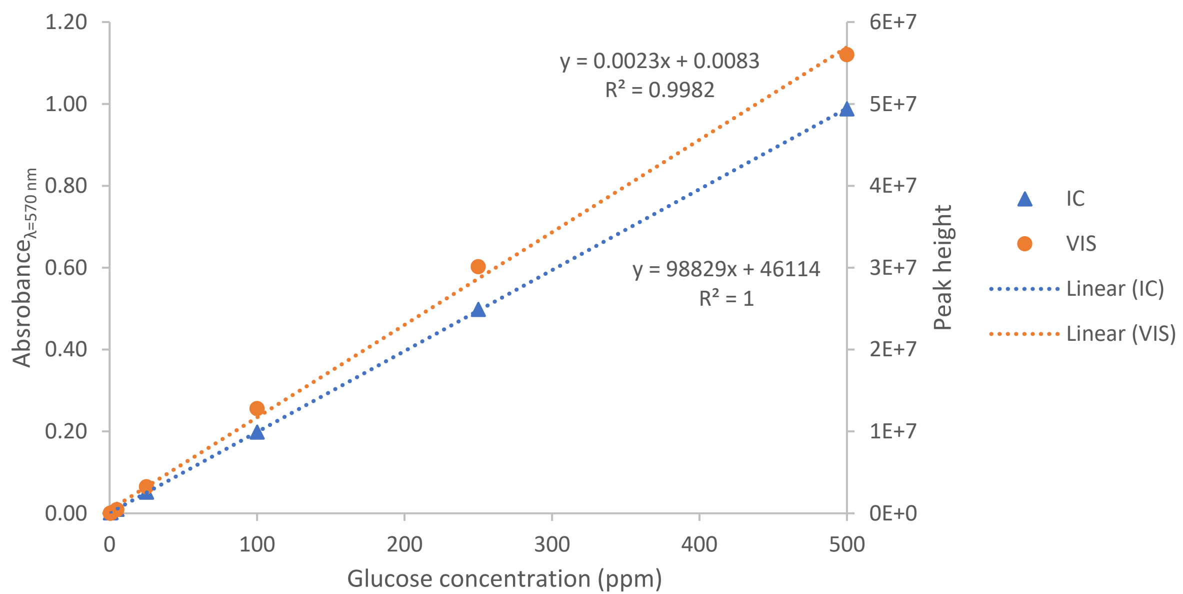

Figure 4.6. Calibration curves for glucose concentrations from IC (blue; y = 98829x + 46114; R^2 = 1) and from spectroscopy readings (orange; y = 0.0023x + 0.0083; R^2 = 0.9982).

The difference between calculated values of glucose concentration measured from IC and spectroscopy is represented visually in Figure 4.7, along with the standard deviations of each treatment with each method.

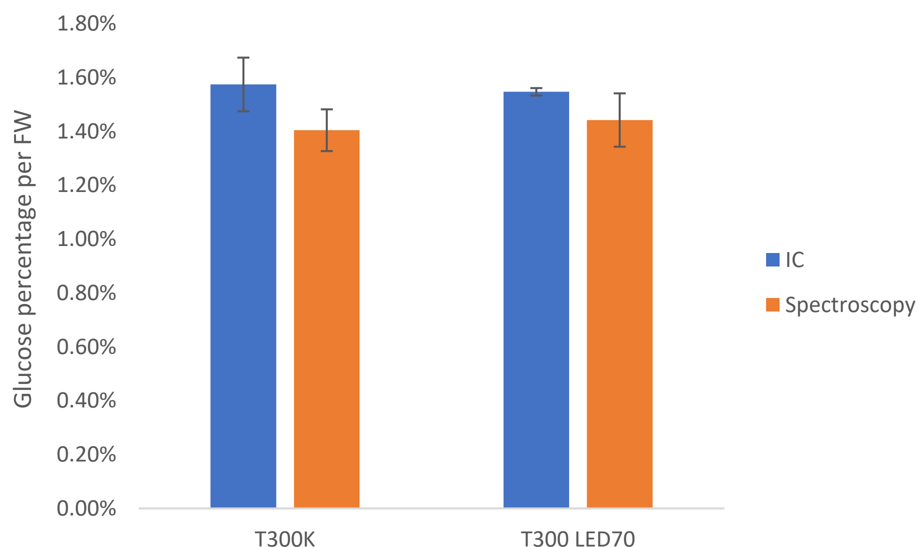

Figure 4.7. Relationship between IC and spectroscopy results for two light treatments expressed in glucose percentage per FW. Error bars show standard deviation (n=3).

In summary, IC and spectroscopy results show a linear correlation at glucose concentrations between 0.5 and 500 ppm and are comparable methods. However, IC shows a stronger linear correlation among responses than spectroscopy (Figure 4.6), along with smaller variation among samples.

5. Discussion

5.1. Basic quality measurements

Basic quality measurements were carried out as a preliminary screening of differences between light treatments and harvests. This was ultimately meant to narrow down the data so in-depth chemical analyses could be performed on the most relevant treatments. Harvest 3 showed the largest differences in SSC both between top lights and between control and LED. Since SSC is directly correlated with sugar concentration, harvest 3 seemed to be the most promising group of samples to produce valuable results.

5.2. IC analysis

IC was used to analyze relevant data determined from the results of basic quality measurements. This method was chosen to be the best option for analysis of all light treatments in harvest 3. The data obtained from IC analyses show significant differences in glucose or fructose concentrations between the light treatments tested.

Interestingly, ANOVAs performed with dry weight data versus fresh weight data gave different results. There were significant differences in sugar concentrations between treatments when considering only the fresh weight, and no such differences when considering the dry weight. This suggests that a dilution effect occurred, and possibly some fruits that had similar DMC had very different water content and FWs. This is important when considering the flavor of a tomato because dilution can affect taste and perceived sweetness.

Based on these results, it can be recommended to Norwegian farmers that supplemental top light and LEDs, in addition to natural sunlight, makes a significant difference on the quality of the tomatoes to a certain extent. From the statistical analyses performed, it seems that T263 with LED70 has a positive impact in sugar concentrations, thus tomato quality. However, this seems to be the extent to which the supplemental light can increase sugar concentrations.

No statistical difference was found between T263 and T300, or between LED70 and LED140, which suggests that increasing sugar content with increasing supplemental light reaches a plateau at some point. Therefore, it can be recommended to use this moderate amount of supplemental light to achieve higher quality tomatoes, so as not to waste energy and money with unnecessary amount of light.

A probable reason for supplemental light positively affecting glucose and fructose concentration can be based on two biological processes occurring within the plant. Energy produced by the light reactions of photosynthesis fuel the reactions that convert CO_2 to glucose, which is converted to sucrose for transport between plant tissues (Beckles et al., 2012), which indirectly means more light equates to more sugars. Additionally, sucrose is transported into the cells during fruit development and is subsequently cleaved, by enzymatic reaction, into glucose and fructose (Figure 2.1). An increase in glucose and fructose concentrations in higher energy light treatments might suggest an influx of sucrose being transported into the fruit, an increase in enzymatic activity, or both.

According to the literature (Pereira da Costa and Conte-Junior, 2015), gradient elution has been ideal in IC analysis of carbohydrates, rather than isocratic elution, because it is meant to give a better separation of compounds. A better separation of compounds could mean the ability to analyze more than only glucose and fructose on IC and could mean a more accurate quantification of glucose and fructose. Gradient elution would have been used for this experiment had the instrumentation been available. At the very least, performing experiments to compare gradient to isocratic elution would have given interesting additional data for this project.

5.3. GC-MS analysis

GC-MS was performed both to be compared to other method for glucose analysis, and to semi-quantitatively analyze seven different compounds thought to be present in tomato fruits.

The GC-MS data show that there is no statistical difference between light treatments for any of the analytes tested (Table 4.1). This is contrary to what was expected based on the results of basic quality measurements, which showed a significant difference in SSC between control and LED 70 in harvest 3. This leads to the suggestion that the SSC difference observed was

due to a variation in a compound that was not analyzed in this study, possibly not a sugar. This is contrary to what literature suggests. Davies & Hobson (1981) report that glucose and fructose make up nearly half of total DMC of a tomato fruit, so it is reasonable to say there is a high likelihood that differences observed in SSC values can be directly correlated with glucose and fructose concentrations.

However, GC-MS instrumentation did not behave ideally. This is shown by a low sensitivity, which is uncharacteristic for GC-MS (Chapter 2.6). Possible reasons for this could include system contamination, or nonoptimal parameters in the operation of the instrument. Additionally, the sample preparation for GC-MS is much more involved than for other methods used in this study and can give rise to many more human errors. While GC-MS has many advantages in this type of analysis (Chapter 2.6), it seems it was not stable in the context of this study so it was not chosen as the main analysis method.

5.4. Spectroscopic analysis

Absorption spectroscopy was performed on two select treatments to compare the use of the method to IC and GC-MS.

Data obtained from spectroscopic analyses showed no significant difference between T300 K and T300 LED70, just as observed by GC-MS analysis. This leads to the same belief previously mentioned: that differences in SSC, which were initially meant to predict differences in glucose and fructose concentrations, could have been due to another compound not analyzed in this method, or even study. The unknown compound could possibly be sucrose that had not been metabolized due to decreased enzymatic activity in the cell.

5.5. Method comparison

The glucose results from IC were quite comparable with those from spectroscopy (Figure 4.5). Each method shows a similar correlation between treatments T300K and T300 LED70, but spectroscopy data show a larger standard deviation than those of IC (Figure 4.7). Based on these results, and previous studies showing the stability and suitability of IC for sugar analysis in food samples (Pereira da Costa and Conte-Junior, 2015), IC was determined to be a better method to use for these analyses.

GC-MS could not be compared in the same way, as the instrument did not behave as it normally should. Sensitivity was greatly decreased and there was evidence of analyte carry-over between samples and standards. However, it can be concluded that with regards to sample preparation, IC prevails over GC-MS. The derivatization required to prepare samples for GC-MS analysis is timely and costly. GC-MS has an advantage over IC in that many more compounds can be analyzed at once, but when looking at only a few analytes IC seems to be the best choice.

5.6. Future aspects

In future considerations of this study, the most important aspect would be to make necessary adjustments to the GC-MS instrument to obtain comparable and reliable results. It is likely that GC-MS could be a better method to analyze sugars than IC, as has been found in the literature, but has not been shown by this study.

Another important piece of the chemical analysis in this study was the type of elution used in IC. Previous studies have shown that gradient elution is the ideal way to analyze sugars in food samples (Pereira da Costa and Conte-Junior, 2015). Unfortunately, limitations in our instruments did not allow this to be utilized. Although, a comparison of values obtained from isocratic elution and gradient elution would be interesting data to include in method development.

Additionally, a taste test would have been very useful with the data produced from this study. It is possible that even if sugar concentration varies, perceived sweetness does not necessarily change (Dzakovich et al., 2015). This could be related to fructose having a sweeter taste than glucose, which could make the perceived sweetness of a tomato depend more on the ratio between the two sugars. Data acquired from a panel of taste testers could do very well in further clarifying if supplemental light, and to what extent, is worth the money and energy.

6. Conclusion

Greenhouse technology is becoming increasingly important as demand for food production continues to rise. Tomatoes have been long studied for their flavor components and the potential to manipulate those components. This study brought these two important points of focus together to find a way greenhouse technology, namely supplemental lighting, can

positively impact the flavor and quality of tomato fruits. It aimed to uncover differences in glucose and fructose concentrations of tomato fruits grown under different lighting conditions in greenhouses, and to recommend an optimal treatment to Norwegian farmers to obtain higher quality tomatoes in the winter season.

After method comparison of three different methods, IC, absorption spectroscopy, and GC, it was found that IC suited this study the best in the analysis of glucose and fructose concentration in tomato fruits. Further analysis with IC showed significant differences between light treatments. The ideal light treatment from this study is shown to be HPS top light of 263 W/m^2 with LED 70. This treatment produces higher quality tomatoes with higher concentrations of glucose and fructose and can be recommended to Norwegian farmers.

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8. Appendix

Table 8.1. SSC data in Brix from each treatment in each harvest, including exact harvest date. SD = standard deviation (n=3).

Harvest No.	Treatment	SSC Mean ± SD (°Brix)	Harvest Date
H1	T176 K	4.93 ± 0.06	5-Dec-18
	T176 LED70	4.97 ± 0.15	5-Dec-18
	T176 LED140	4.80 ± 0.17	5-Dec-18
	T263 K	5.13 ± 0.06	5-Dec-18
	T263 LED70	5.13 ± 0.06	5-Dec-18
	T300 K	5.10 ± 0.00	7-Dec-18
	T300 LED70	5.23 ± 0.12	10-Dec-18
H2	T176 K	4.67 ± 0.12	17-Dec-18
	T176 LED70	4.80 ± 0.10	17-Dec-18
	T176 LED140	4.97 ± 0.12	17-Dec-18
	T263 K	5.17 ± 0.06	17-Dec-18
	T263 LED70	5.20 ± 0.10	17-Dec-18
	T300 K	5.07 ± 0.06	14-Dec-18
	T300 LED70	5.30 ± 0.00	14-Dec-18
H3	T176 K	4.40 ± 0.00	7-Jan-19
	T176 LED70	4.77 ± 0.06	7-Jan-19
	T176 LED140	4.90 ± 0.10	7-Jan-19
	T263 K	5.10 ± 0.20	7-Jan-19
	T263 LED70	4.87 ± 0.06	7-Jan-19
	T300 K	5.10 ± 0.17	11-Jan-19
	T300 LED70	5.33 ± 0.12	11-Jan-19

Table 8.2. Raw fresh weight and dry weight (DW) data from 7 light treatments sampled in triplicate, with calculated glucose and fructose concentrations per dry weight and per fresh weight based on IC data.

Sample	Treatment	FW (g)	DW (mg)	% gluc of DW	%fruct of DW	% gluc of FW	% fruct of FW	gluc:fruct ratio
73	T263 LED70	0.56	35.76	22.6%	24.8%	1.46%	0.0160	0.911
74	T263 LED70	0.56	33.18	24.2%	26.4%	1.45%	0.0158	0.917
75	T263 LED70	0.56	35.21	22.8%	24.1%	1.43%	0.0151	0.946
76	T263K	0.55	34.78	22.8%	25.2%	1.44%	0.0159	0.905
77	T263K	0.55	35.52	24.8%	26.4%	1.61%	0.0172	0.937
78	T263K	0.46	30.80	25.3%	26.3%	1.69%	0.0176	0.959
79	T176 LED70	0.57	33.38	23.2%	25.7%	1.35%	0.0150	0.902
80	T176 LED70	0.48	30.22	21.6%	22.7%	1.35%	0.0142	0.951
81	T176 LED70	0.58	32.05	24.7%	27.3%	1.36%	0.0150	0.907
82	T176 LED140	0.46	25.38	25.6%	28.8%	1.42%	0.0159	0.889
83	T176 LED140	0.48	26.78	24.5%	27.3%	1.36%	0.0151	0.900
84	T176 LED140	0.54	31.83	24.7%	25.8%	1.47%	0.0153	0.959
85	T176K	0.57	29.12	22.7%	26.2%	1.15%	0.0133	0.865
86	T176K	0.53	26.67	22.8%	25.5%	1.15%	0.0129	0.895
87	T176K	0.46	23.45	23.3%	26.4%	1.18%	0.0134	0.883
133	T300K	0.52	32.51	23.3%	25.2%	1.47%	0.0159	0.924
134	T300K	0.49	33.01	23.6%	25.0%	1.59%	0.0169	0.943
135	T300K	0.57	38.97	24.4%	25.8%	1.66%	0.0176	0.944
145	T300 LED70	0.55	36.93	23.3%	26.2%	1.56%	0.0176	0.889
146	T300 LED70	0.52	34.47	23.0%	26.1%	1.54%	0.0175	0.883
147	T300 LED70	0.53	34.31	23.5%	25.1%	1.54%	0.0164	0.938

Table 8.3. Pairwise comparison results for the effects of top light, LED, and harvest (H) on SSC, based on 3-way ANOVA followed by Holm-Sidak tests. Comparisons with significant differences are marked with an asterisk (*).

Comparison for factor:	Comparison	Diff of Means	t	P	P<0.050
Top Light	T300 vs. T176*	0.433	13.122	<0.001	Yes
	T263 vs. T176*	0.344	10.43	<0.001	Yes
	T300 vs. T263*	0.0889	2.692	0.011	Yes
LED	LED70 vs. CONTROL*	0.104	3.846	<0.001	Yes
Harvest	1 vs. 3*	0.156	4.71	<0.001	Yes
	2 vs. 3*	0.106	3.196	0.006	Yes
	1 vs. 2	0.05	1.514	0.139	No
Top Light within H1	T300 vs. T176*	0.217	3.788	0.002	Yes
	T263 vs. T176*	0.183	3.205	0.006	Yes
	T300 vs. T263	0.0333	0.583	0.564	No
Top Light within H2	T263 vs. T176*	0.45	7.867	<0.001	Yes
	T300 vs. T176*	0.45	7.867	<0.001	Yes
	T263 vs. T300	0	0	1	No
LED within H2	LED70 vs. CONTROL*	0.133	2.855	0.007	Yes
Top Light within Control-H3	T263 vs. T176*	0.7	8.654	<0.001	Yes
	T300 vs. T176*	0.7	8.654	<0.001	Yes
	T263 vs. T300	0	0	1	No
Top Light within LED70-H3	T300 vs. T176*	0.567	7.005	<0.001	Yes
	T300 vs. T263*	0.467	5.769	<0.001	Yes
	T263 vs. T176	0.1	1.236	0.224	No
LED within T176-H3	LED70 vs. CONTROL*	0.367	4.533	<0.001	Yes

Table 8.4. Pairwise comparison results for the effects of LED and harvest (H) on SSC, based on 2-way ANOVA followed by Holm-Sidak tests. Comparisons with significant differences are marked with an asterisk (*).

Comparison for factor:	Comparison	Diff of Means	t	P	P<0.050
LED	LED140 vs. CONTROL*	0.222	4.33	0.001	Yes
	LED70 vs. CONTROL*	0.178	3.464	0.006	Yes
	LED140 vs. LED70	0.0444	0.866	0.398	No
Harvest	1 vs. 3*	0.211	4.114	0.002	Yes
	2 vs. 3	0.122	2.382	0.056	No
	1 vs. 2	0.0889	1.732	0.1	No
Harvest within Control	1 vs. 3*	0.533	6	<0.001	Yes
	1 vs. 2*	0.267	3	0.015	Yes
	2 vs. 3*	0.267	3	0.008	Yes
Harvest within LED70	1 vs. 3	0.2	2.25	0.107	No
	1 vs. 2	0.167	1.875	0.148	No
	2 vs. 3	0.0333	0.375	0.712	No
Harvest within LED140	2 vs. 1	0.167	1.875	0.214	No
	3 vs. 1	0.1	1.125	0.475	No
	2 vs. 3	0.0667	0.75	0.463	No
LED within H1	LED70 vs. LED140	0.167	1.875	0.214	No
	CONTROL vs. LED140	0.133	1.5	0.279	No
	LED70 vs. CONTROL	0.0333	0.375	0.712	No
LED within H2	LED140 vs. CONTROL*	0.3	3.375	0.01	Yes
	LED140 vs. LED70	0.167	1.875	0.148	No
	LED70 vs. CONTROL	0.133	1.5	0.151	No
LED within H3	LED140 vs. CONTROL*	0.5	5.625	<0.001	Yes
	LED70 vs. CONTROL*	0.367	4.125	0.001	Yes
	LED140 vs. LED70	0.133	1.5	0.151	No

Table 8.5. Pairwise comparison results for the effects of top light and LED on glucose per fresh weight, based on 2-way ANOVA followed by Holm-Sidak tests. Comparisons with significant differences are marked with an asterisk (*).

Comparison for factor:	Comparison	Diff of Means	t	P	P<0.050
Top Light	T300 vs. T176*	0.00303	7.946	<0.001	Yes
	T263 vs. T176*	0.00257	6.723	<0.001	Yes
	T300 vs. T263	0.000467	1.222	0.245	No
LED	LED70 vs. CONTROL	0.000111	0.356	0.728	No
LED within T176	LED70 vs. CONTROL*	0.00193	3.581	0.004	Yes
LED within T263	CONTROL vs. LED70*	0.00133	2.47	0.03	Yes
LED within T300	CONTROL vs. LED70	0.000267	0.494	0.63	No
Top Light within Control	T263 vs. T176*	0.0042	7.779	<0.001	Yes
	T300 vs. T176*	0.00413	7.656	<0.001	Yes
	T263 vs. T300	0.0000667	0.123	0.904	No
Top Light within LED70	T300 vs. T176*	0.00193	3.581	0.011	Yes
	T300 vs. T263	0.001	1.852	0.17	No
	T263 vs. T176	0.000933	1.729	0.109	No

Table 8.6. ANOVA results from a test of the effects of top light and LED on glucose per dry weight, based on a 2-way ANOVA.

Source of Variation	DF	SS	MS	F	P
Top Light	2	0.000152	7.62E-05	0.846	0.453
LED	1	9.34E-05	9.34E-05	1.037	0.329
Top Light x LED	2	0.000134	6.69E-05	0.743	0.496
Residual	12	0.00108	9.01E-05		
Total	17	0.00146	8.59E-05		

Table 8.7. Pairwise comparison results for the effects of top light and LED on fructose per fresh weight, based on 2-way ANOVA followed by Holm-Sidak tests. Comparisons with significant differences are marked with an asterisk (*).

Comparison for factor:	Comparison	Diff of Means	t	P	P<0.050
Top Light	T300 vs. T176*	0.00302	8.144	<0.001	Yes
	T263 vs. T176*	0.0023	6.209	<0.001	Yes
	T300 vs. T263	0.000717	1.935	0.077	No
LED	LED70 vs. CONTROL	0.000211	0.698	0.498	No
LED within T176	LED70 vs. CONTROL*	0.00153	2.927	0.013	Yes
LED within T263	CONTROL vs. LED70*	0.00127	2.418	0.032	Yes
LED within T300	LED70 vs. CONTROL	0.000367	0.7	0.497	No
Top Light within Control	T263 vs. T176*	0.0037	7.063	<0.001	Yes
	T300 vs. T176*	0.0036	6.872	<0.001	Yes
	T263 vs. T300	0.0001	0.191	0.852	No

Top Light within LED70	T300 vs. T176*	0.00243	4.645	0.002	Yes
	T300 vs. T263*	0.00153	2.927	0.025	Yes
	T263 vs. T176	0.0009	1.718	0.111	No

Table 8.8. Pairwise comparison results for the effects of LED on glucose per fresh weight, based on 1-way ANOVA followed by Holm-Sidak tests. Comparisons with significant differences are marked with an asterisk (*).

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
LED140 vs. Control*	0.257	9.384	<0.001	Yes
LED70 vs. Control*	0.193	7.068	<0.001	Yes
LED140 vs. LED70	0.0633	2.315	0.06	No

Table 8.9. Pairwise comparison results for the effects of LED on glucose per fresh weight, based on 1-way ANOVA followed by Holm-Sidak tests. Comparisons with significant differences are marked with an asterisk (*).

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
LED140 vs. Control*	0.00223	7.011	0.001	Yes
LED70 vs. Control*	0.00153	4.813	0.006	Yes
LED140 vs. LED70	0.0007	2.197	0.07	No