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Abstract

Tomato (*Solanum lycopersicum*) is one of the most consumed vegetables in the world. It is a source of antioxidants, minerals and vitamins, and has many beneficial effects on health.

In the last decades, the taste properties of commercially produced tomatoes have declined as a consequence of breeding for high yield, harvesting at the green immature stage and improper post-harvesting practices. Several recent studies therefore focus on gaining information how to reattain high quality in terms of organoleptic properties. A challenge in norwegian tomato production is lack of light, even in the summer months. To solve this problem producers utilize supplementary lighting to provide enough irradiation for the tomato plants. Another aspect that is becoming important in food production is environmental pollution. The conventional hydroponic system has the disadvantages of using high amounts of water and releasing unused mineral fertilizers into the environment. The organoponic system is a new approach where organic fertilizer is used and recirculated, which allows for a sustainable production of fresh vegetables.

It is well known that taste of tomato fruits is affected by a combination of organic acids, sugars and volatile compounds. In this study, the content of organic acids and quality of tomatoes as a function of different lighting and nutrients were investigated. The effect of organic fertilizer and growth promoting bacteria on content of organic acids and quality was compared to standard mineral nutrient solution. In addition, the influence of supplementary inter-row and top light intensities on the quality and content of organic acids in tomato fruits was examined.

The results showed that quality of tomatoes is affected by both light and nutrients. LED inter-row lighting had a positive effect on the quality of tomatoes at lower top light (HPS lamps) intensities. Tomatoes grown in standard mineral nutrient solution had higher quality (higher TTA and SSC) than tomatoes grown with organic fertilizer. However, tomatoes grown with organic fertilizer had the highest levels of vitamin C. The organoponic system is a new promising approach with potential for improvements. It is environmentally friendly method that deserves further attention and research.

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Stavanger, June 2019

Lea Zalar Pettersen

Abbreviations

ANOVA	Analysis of variance
CAE	Citric acid equivalents
DHA	Dehydroascorbic acid
DW	Dry weight
FW	Fresh weight
GABA	Gamma - aminobutyric acid
GC	Gas chromatography
HPS lamp	High pressure sodium lamp
EI	Electron ionization
LED	Light emitting diode
MS	Mass spectroscopy
m/z	Mass to charge ratio
PCA	Principal component analysis
PGPB	Plant growth promoting bacteria
ppm	Parts per million
SSC	Soluble solid content
TIC	Total-ion chromatogram
TTA	Total titratable acidity

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1 INTRODUCTION

1.1 Background and scope of the assignment

Tomato (*Solanum lycopersicum)* is one of the most widely produced and consumed vegetables in the world. In the western countries, tomato is on the second place after potatoes among vegetables in terms of production and consumption. Tomato is an important part of the Mediterranean diet which is known to have many benefits for the health. Numerous epidemiological studies have been carried out in which high intake of the tomato products was shown to be correlated with reduced risk for various types of cancer and cardiovascular diseases. *(Borguini and Torres 2009; Giovannucci 2002; Weisburger 2002; Willcox et. al.2003)*

China and India are the biggest producers of tomatoes. Northern Europe (Norway, Netherland, Belgium, United Kingdom, Ireland) have much lower production measured by quantity, but they have the highest yield. This is despite the fact that climate is not favourable and that areas dedicated for production are small. The reason for high yield is that northern countries produce tomatoes in greenhouses, where the conditions are highly controlled and adapted to tomato production *(Bergougnoux 2014).*

Almost all production of tomatoes (90%) in Norway is located in Rogaland, where mild climate with stable temperatures throughout the year allows year-round greenhouse production of tomatoes *(Verheul 2019)*. In 1995, the share of Norwegian tomatoes on the market was 70 %. Since then, the consumption increased, while the producers have not managed to follow the consumer's demand. The average consumption of fresh tomatoes in 2017 in Norway was 6.99 kg/capita/year, where only 36% of the tomatoes were produced in Norway*. (Haug 2018; Rebnes and Angelsen 2017)*

Availability of Norwegian tomatoes on the market is not stable throughout the year (Figure1.1). In the winter, only 5% of the tomatoes in the market are produced in Norway while the rest are imported from Spain, the Netherlands and other countries. In the summer the situation is reversed, where Norwegian tomatoes dominate the market. The reason for

this is that in the summer trade tariffs for tomatoes are significantly higher as in the winter months in order to protect domestic producers. *(Realfsen 2005; "Tomater og klimaavtrykk | Norsk Gartnerforbunds energiside")*

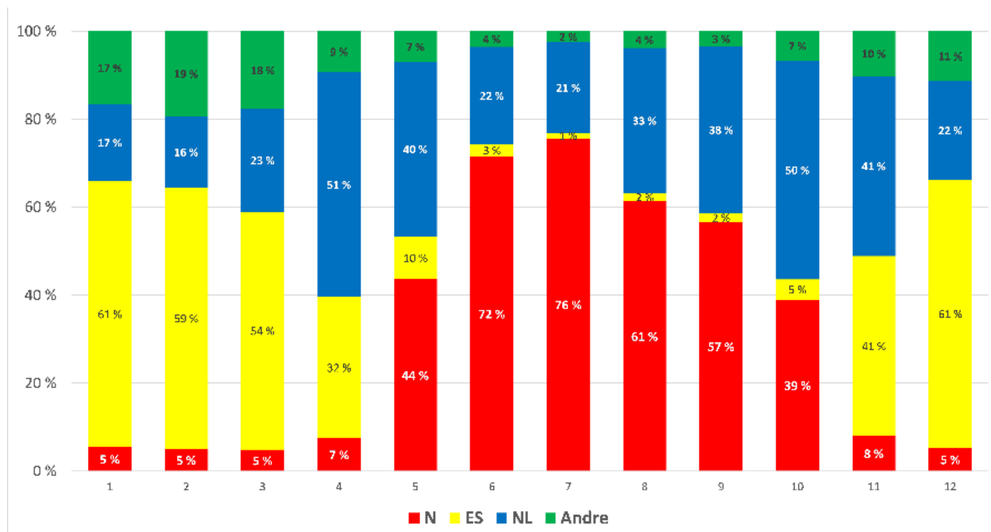

***Figure 1.1** Norwegian consumption of tomatoes per month from different origin, where N stands for Norwegian tomatoes, ES for Spanish tomatoes, NL for tomatoes from the Netherlands and Andre for tomatoes from other countries. ("Tomater Og Klimaavtrykk | Norsk Gartnerforbunds Energiside")*

The intake of tomatoes and vegetables in general in Norway is not very high, however according to The Norwegian Directorate of Health the consumption of vegetables in Norway increased by around 25% in the last ten years *(Meltzer et al. 2018)*.

A common consumer's complain regarding tomatoes is that flavour of the commercially available tomatoes has declined in the last decades compared to old heirloom varieties. One of the reasons for this is that most of the recent breeders have been focused on traits as high yield, disease resistance and high firmness. All these qualities were in favour for easier transportation and long-term storage, but as the consequence, the flavour deteriorated. Another practice that leads to degradation of flavour is harvesting tomatoes at the green immature stage and ripening them by the induction of ethylene. This again facilitates the transport and storage while the taste and aroma are affected in a negative way *(Bennett 2012)*. Nevertheless, in recent years a lot of research has been conducted in order to understand how to recover a good flavour through molecular breeding *(Tieman et al. 2017)* and investigate best harvesting and post-harvesting practices for tomato fruits *(Gautier et al. 2008; Verheul et al. 2015)*.

One of the challenges that Norwegian tomato producers experience is the lack of light, even in the summer months. To address this problem a supplementary LED lighting can be utilized.

Another aspect that many producers as well as consumers recognize as important is the environmental pollution. It has been shown that norwegian tomatoes have lower carbon footprint as imported spanish tomatoes. Moreover, water consumption per kilogram of produced tomatoes in Spain is 60 liters, while in Norway it is 6 times lower *(Verheul 2019)*. However, there is a potential for reducing pollution of mineral fertilizers and waste of water by using the organic fertilizer in a recirculation system, which would lead to a more sustainable production of fresh vegetables.

The objective of the master thesis is to find out if and how different inter-row and top light intensities, as well as organic fertilization with added growth promoting bacteria affects the quality of tomato fruits grown in the greenhouse. Main focus is to analyse contents of organic acids in tomatoes, as it is known that acids along with sugars and volatile compounds affect the organoleptic properties of tomatoes. Information gained by this thesis may be beneficial for local tomato producers who aim to increase quality of the tomatoes as well as make production more sustainable. The thesis is a part of the Biofresh project led by NIBIO (Norwegian Institute of Bioeconomy Research) *("Bioeconomic production of fresh greenhouse vegetables in Norway")*.

1.2 General about tomato

1.2.1 Brief history of tomato

Origin of wild tomato species is a region between western South America from Ecuador to northern part of Chile, as well as the Galapagos Islands. It is believed that tomatoes were first domesticated by Aztecs in Mexico around 500 BC. In the 16th century, tomato was introduced in Europe by Spanish conquistador Hernán Cortés, who got seeds of a small yellow tomato in Mexico and brought them to Spain (Figure 1.2). In the following years, tomato was spread also to other parts of Europe, although in some places at the beginning just for a decoration because of its beauty. Today, tomato is spread all over the world and is

one of the most important fresh vegetables in the industrialized world. *(Bergougnoux 2014; Benton 2007; “Tomato Facts - Interesting Facts about Tomatoes”)*

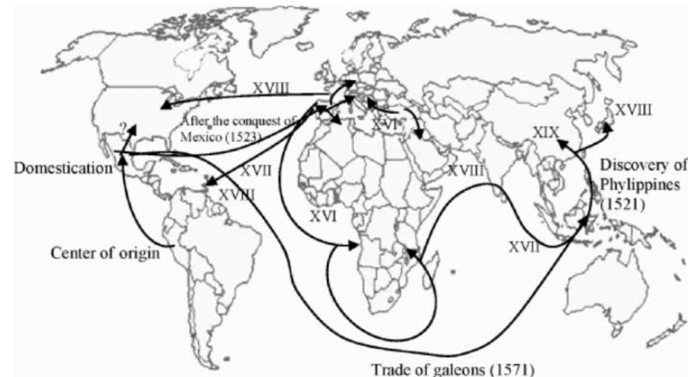

Figure 1.2 *Origin and spreading of tomatoes throughout the world. (Díez and Nuez 2008)*

1.2.2 Tomato fruit characteristics

There exist hundreds of tomato varieties with varying morphological and sensory characteristics of fruits (Figure 1.3). Tomato is cultivated and used as a vegetable, even though it is botanically classified as a fruit berry. It has several fruit characteristics: it evolves from the ovary of the plant, possesses pericarp walls and skin which give fleshiness, and has several seeds inside *(Bergougnoux 2014)*. Fruit shape and size is affected by the number of cavities containing seeds (locules) *(Muños et al. 2011)*.

Figure 1.3 *Different varieties of tomatoes differ in colour, shape, size and flavour. All tomatoes on the picture were available in the Norwegian supermarket.*

1.2.3 Fruit development

Tomato has been widely used as a model for research of fleshy fruit development and ripening *(Kimura and Sinha 2008)*. In first stages of the development process, fruits serve as a protection for seeds from environmental impacts and predators. During the ripening process, extensive transformation of metabolites (organic acids, sugars, volatile compounds…) occurs, which contributes to flavour, aroma and colour of fruits. This makes them more attractive for animals that eat fruits and disseminate the ingestible seeds, and in this way promote their dispersal in the surrounding environment. *(Rodríguez et al. 2013; Srivastava and Handa, 2005)*

The tomato fruit development is regulated by endogenous and external environmental signals and can be divided into four phases (Figure 1.4).

Phase I: Floral development, pollination, fertilization and fruit set takes place.

Phase II: Rapid cell division resulting in a large increase in number of pericarp cells. In this phase most of the fruit cells are established. However, the fruit growth in this phase is slow and reaches only about 10% of the final fresh weight. *(Pesaresi et al. 2014; Srivastava and Handa 2005)*

Phase III: Cell expansion leading to significant increase in weight and attainment of the final size of the fruit. This depends on the genotype and the environmental conditions. *(Chevalier 2007; Pesaresi et al. 2014)*

Phase IV: Extensive metabolic changes start, while the growth of the fruit is slowed. Phase IV can be further divided into breaking (BR) and ripening (RR) stages. Breaking stage is characterised by transformation of chloroplasts into chromoplasts. This can be observed by change in colour from green to yellow-orange due to carotenoid and lycopene accumulation and chlorophyll degradation. *(Pesaresi et al. 2014)*

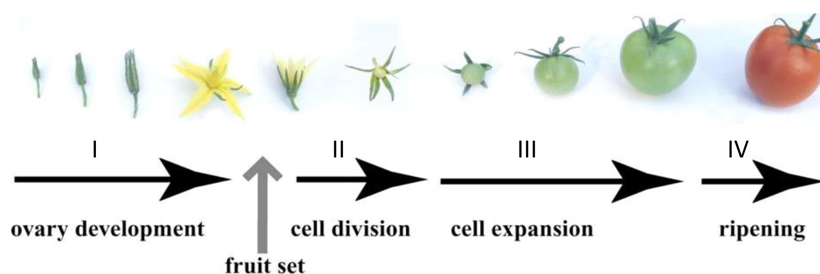

Figure 1.4 *Four phases of tomato fruit development ("Cyclus van Een Tomaat")*

1.2.4 Metabolism of organic acids during fruit development and ripening

Citric and malic acid are major acids present in tomatoes. Metabolism and accumulation of these two acids have therefore a main role in acidity of tomato fruits. Studies have shown that physiological mechanisms controlling the metabolic pathways of citric and malic acid are controlled by genetic *(Fortes et al. 2017; Famiani et al. 2005; Schauer et al. 2006)* and environmental factors *(Verheul 2012; Gautier et al. 2008)*.

Metabolism of citric and malic acid in fleshy fruits takes place in several pathways (Figure 1.5). First step in formation of organic acids is carboxylation of phosphoenolpyruvate (PEP), that is formed from glucose imported from leaves through glycolysis pathway. This occurs in cytosol and results in the synthesis of dicarboxylates, malate and oxaloacetate (OAA). The opposite process, degradation of organic acids, occurs through decarboxylation of malate and OAA in cytosol that allows the synthesis of PEP. This leads to gluconeogenesis, a metabolic pathway that generates glucose. Gluconeogenesis arises during ripening when acidity of the fruit decreases and sugars accumulate. Malate and OAA in cytosol can be converted into citrate or other dicarboxylates through the tricarboxylic acid (TCA) cycle and the glyoxylate cycle. TCA cycle takes place in mitochondria and is a part of cellular respiration. It involves sequence of reactions involving different acids that result in oxidation of pyruvate into CO_2. In glyoxylate cycle, acetyl-CoA is converted into succinate. Succinate can be further consumed in TCA cycle and converted into malate which can take part in gluconeogenesis and generate glucose. Citrate that is synthesized in TCA cycle can be degraded by gamma-aminobutyrate synthesis pathway (also called GABA shunt) and acetyl-CoA catabolism. Both pathways take place in cytosol and produce dicarboxylic acids which result in decrease of acidity. *(Etienne et al. 2013)*

In the early stages of tomato fruit development organic acids are present in high concentrations which allow the continuous supply of substrates for sustaining the cellular respiration. The levels of sugars in the early stages are low, but increase rapidly during ripening, while content of organic acids is reduced. *(Carrari et al. 2006)*

It is known that carboxylic acids have an important role in the phase of cell expansion and consequently in fruit growth. They affect the osmotic potential that drives water uptake and thus the cell expansion *(Liu et al. 2007)*.

A study conducted by Centeno and his colleagues showed that malate has an important role in metabolism of starch and content of soluble solids in tomato fruits. They demonstrated that lines with high levels of malate resulted in low levels of starch and soluble solid content at the harvest and opposite, lower levels of malate resulted in high levels of these carbohydrates. This indicates that malate is an important regulatory metabolite. *(Centeno et al. 2011)*

The role of organic acids during the development and ripening of fruits is very complex and not yet fully understood. In recent years several studies on different types of fruits indicated that organic acids are important regulatory metabolites together with hormones and not just a consequence of metabolic processes. More knowledge in this field would allow the engineering of organic acid metabolism and improve qualitative and quantitative features of the crops. *(Batista-Silva et al. 2018)*

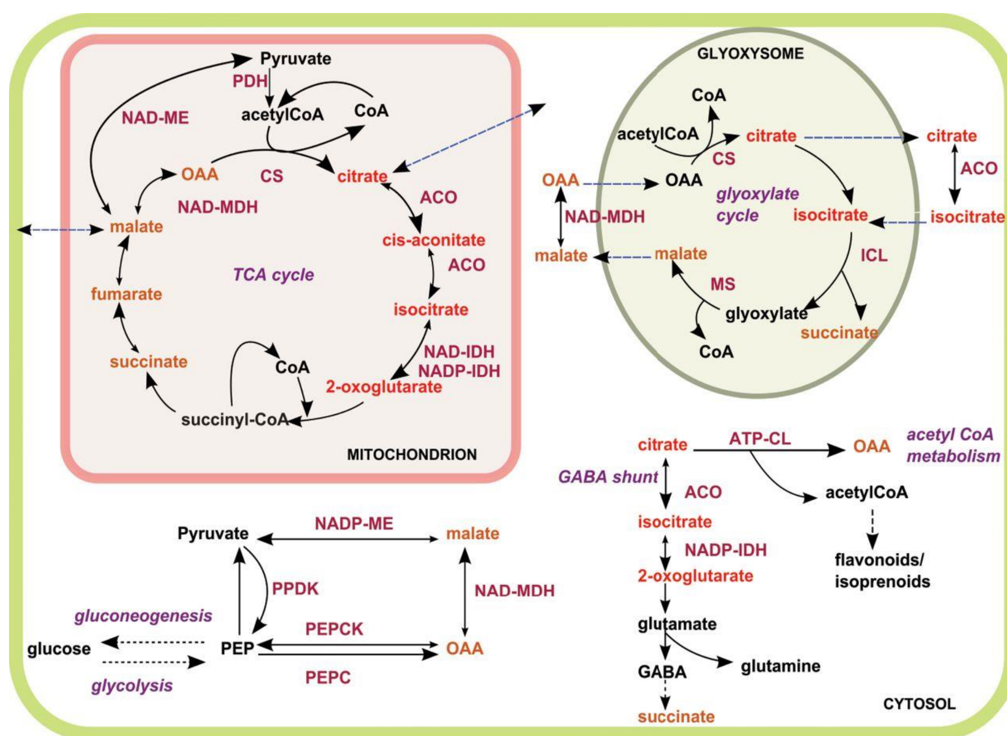

Figure 1.5 *Citric and malic acid metabolic pathways in fruit cells. ACO, aconitase; ATP-CL, ATP-citrate lyase; CS, citrate synthase; ICL, isocitrate lyase; MS, malate synthase; NAD-MDH, NAD-malate dehydrogenase; NAD-ME, NAD-malic enzyme; NAD-IDH, NAD-isocitrate dehydrogenase; NADP-ME, NADP-malic enzyme; NADP-IDH, NADP-isocitrate dehydrogenase; PDH, pyruvate dehydrogenase; PEPC, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PPDK, pyruvate orthophosphate dikinase. The probable direction of reversible reactions is indicated by the large arrow. Dashed blue arrows indicate malate and citrate transport.* (Etienne et al. 2013)

1.3 Quality of tomatoes

Main factors contributing to the quality of tomatoes are organoleptic properties (flavour), health beneficial properties, visual appearance (colour and absence of damages), dry matter content (DMC) and firmness and texture *(Dorais et al. 2010)*.

Fruit flavour is influenced by a combination of taste and aroma. Content of sugars and acids contribute to the taste of tomatoes, while volatile compounds have influence on the aroma *(Klee and Tieman 2013; Malundo et al. 1995; Tieman et al. 2012).* Main acids in the tomato are citric acid, malic acid and glutamic acid (Figure 1.6), with citric acid being the most abundant *(Yilmaz 2001)*. Citric and malic acid affects the tartness of the tomatoes, while glutamic acid contributes to umami taste *(Kurihara 2009)*. There are many more organic acids present in the tomato fruit (ascorbic acid, maleic acid, succinic acid, shikimic acid, fumaric acid, oxalic acid, malonic acid, *et cetera*), but they are present in very small quantities and hence do not affect the taste *(Luengwilai et al. 2012; Zhao et al. 2016)*. Fructose and glucose are present in the highest amounts among sugars. High sugar and high acid content is a combination that gives the most tasteful tomato. If the content of sugars is high and the content of acids low, the taste is perceived as bland. When opposite, low sugar content and high acid content, taste is perceived as tart *("Managing Tomato Taste" 2018)*.

A common way to estimate the content of sugar in the fresh commodities is measuring the level of soluble solid content (SSC), which is expressed in values of °Brix. Soluble solids in tomato consist predominantly of sugars, organic acids, amino acids and pectin. All of this compounds contribute to the °Brix values, but since sugars are present in the highest concentration (approximately 50 %), °Brix is a good estimation of sugar content in the fruits *(Kleinhenz and Bumgarner).*

It is important to mention that lower °Brix value and thus less sugar content does not necessarily mean the lower sweetness of tomato fruit. The reason for this is that some volatile compounds contribute to the perception of sweetness. If those volatiles are present in higher amounts, the tomato fruit taste sweeter regardless of sugar content. *(Klee and Tieman 2013)*

Acidity of fruits is estimated by measuring the total titratable acids (TTA). This estimation is a better predictor of acid's impact on flavour than pH, as it measures the total concentration of acids within a fruit. *(Sadler and Murphy 2010)*

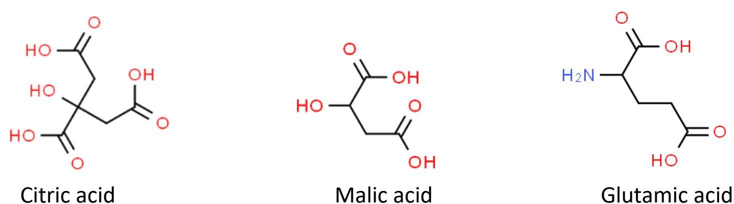

Figure 1.6 *Main organic acids contributing to the taste in tomato fruit are citric acid, malic acid and glutamic acid*. *("Citric Acid | ChemSpider"; "Glutamic Acid | ChemSpider"; "Malic Acid | ChemSpider")*

Colour is one of the first characteristics that affects the initial decision of a consumer to purchase a vegetable or a fruit *(Verheul et al. 2015)*. For each vegetable and fruit, consumers have a preferred colour and for the tomato it is red *(Barrett et al. 2010)*. Recent study conducted by Nofima *(Hansen 2017)*, where the consumer's response to cherry tomatoes of different colours was investigated supports the preference for the red coloured tomatoes.

Firmness of the tomato fruits is important for the consumers as well as for the producers and wholesalers. Norwegian consumers favour tomatoes with high firmness as they are considered to have a better texture. Tomatoes with high firmness are also desired by the wholesalers because of facilitated transport and storage as firm tomatoes are less prone to damage and have a longer shelf life *(Verheul et al. 2015)*.

1.4 Greenhouse production of tomatoes

Tomato is a demanding plant in terms of climate conditions. It is a warm-season crop with optimum daytime growth temperature between 21 °C to 27 °C and night temperature at least 15 °C *(Buschermohle and Grandle)*. Environmental conditions like strong wind can lead to mechanical damage of the plants. Moreover, humid and rainy weather can cause the occurrence and spreading of diseases. Due to the harsh climate and deficiency of natural light in northern latitudes, the field production of the tomatoes is not possible in these regions. As a consequence, commercial tomatoes in the northern countries are produced in

greenhouses, where the environmental and agronomic factors can be controlled and automated *(Erba et al., 2013; "Tomato Cultivation | Tomato Climatic Requirement")*. The modern greenhouse facilities with control of air temperature, humidity, CO_2 levels, air circulation and nutrient composition provide optimal growing conditions for the plants, as well as they serve as a protection against harmful weather events, insects and diseases that can spread out in the field. This allows for year-round production, increases yield and quality and reduces need for use of chemicals and pesticides. *(Brown 2015)*

Despite the fact that greenhouses are made of glass transparent to sunlight, natural solar radiation does not always provide a sufficient quantity of light energy for a commercial growing of crops. The use of artificial supplemental lighting to compensate for the naturally low solar radiation has therefore become widely utilized practice, especially in the northern countries and during the winter period. *("Dutch Greenhouse Technology | DutchGreenhouses®"; Dzakovich et al. 2015)*

Traditional and the most common way of providing supplemental lighting in greenhouses is from the overhead high-pressure sodium (HPS) lamps (Figure 1.7). HPS lamps operate at high temperature (≥ 200 °C) and thus generate high radiant heat emission. This can harm plants and to avoid this, the lamps must be placed at the certain distance (at least two meters) above them. In addition, HPS lamps have a high-life cycle cost and significant impact on the environment. Light-emitting diodes (LED) as a source for greenhouse lighting is a new energy-efficient approach. This technology has several advantages over traditional HPS lamps and an extensive research of the influence of LED lighting on the indoor plant cultivation has been going on for over a decade. *(Singh et al. 2015)*

LED is a type of semiconductor diode that can be controlled digitally in order to optimize spectral composition and light intensity. It is known that plants do not absorb all wavelengths of light (white light), but rather selective wavelengths according to their needs. Controlling and optimizing the spectral composition can therefore improve the plant growth and LEDs have a clear advantage over HPS lamps in this *(Yeh and Chung 2009).* In addition, LEDs are also more efficient than HPS lamps. They can convert up to 50% of energy into a usable light and can be placed close to the plants (Figure 1.7) as they do not emit radiant heat. As a comparison, HPS lamps convert only 30% of energy into a usable light. Furthermore, LEDs have a longer lifetime (up to 50 000 hours) compared to HPS lamps

(approximately 20 000 ours). Nevertheless, it needs to be mentioned that implementing a LED lighting system into the greenhouse represents a high capital investment. However, economic analysis has shown that LEDs reduce the cost of electricity significantly, and the investment is returned as profit in long-term operations in commercial greenhouse facilities *(Singh et al. 2015).* In Norway, tomato producer Orre from Klepp has started to use LED lighting technology and this indicates that interest for a sustainable and energy efficient production has also reached the commercial producers in Norway. *(Rogaland fylkeskommune 2016)*

Effect of LED illumination on plants has been subject of many studies in recent years, focusing both on physiological and morphological changes, as well as influence on quality and yield of vegetable crops. Dzakovich and co-workers demonstrated that saving costs by using LED supplemental lighting instead of only traditional HPS lighting does not negatively affect the sensory properties of tomato fruits *(Dzakovich et al. 2015).* Another interesting research was conducted by Wageningen UR Greenhouse in collaboration with Phillips, in which it was shown that LED light irradiation increases levels of L-ascorbic acid (vitamin C) in tomato fruits *(Ntagkas et al. 2016).*

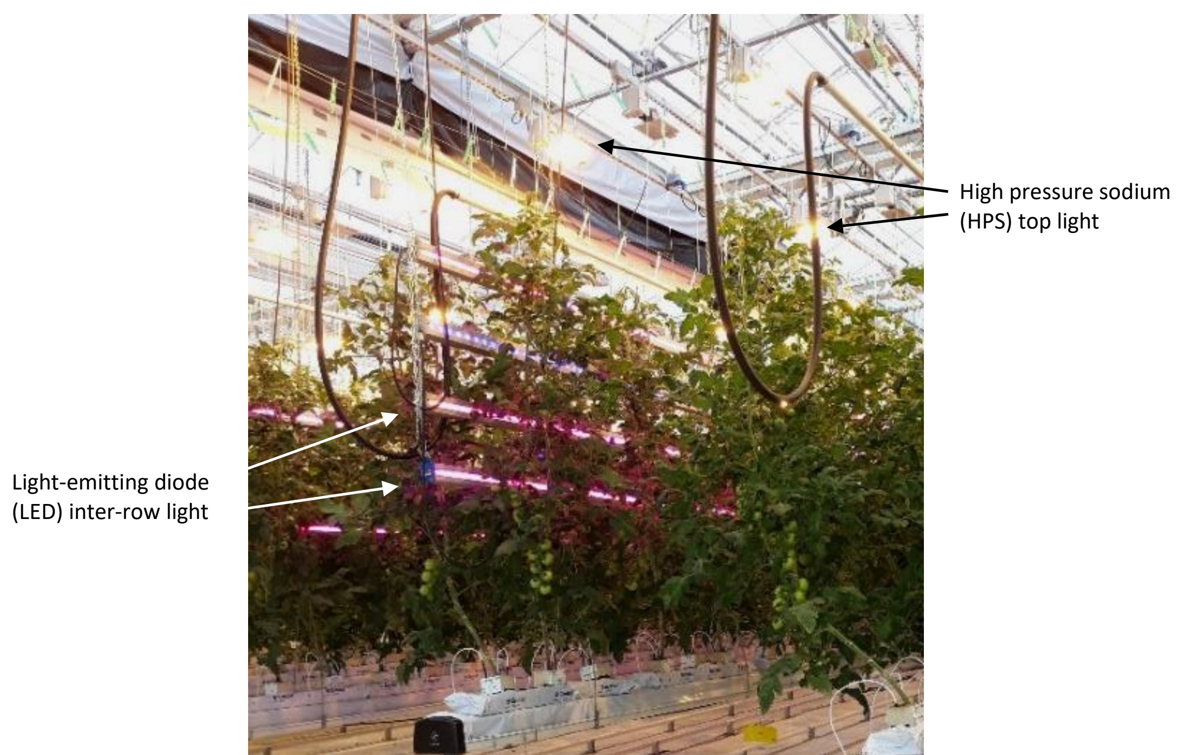

Figure 1.7 *HPS and LED supplemental lighting in Nibio research greenhouse at Særheim.*

Traditional growing of crops in soil has several disadvantages like loss of nutrients, restricted availability of land and excessive use of pesticides. Hydroponic system, a practice of growing plants in a nutrient-rich aqueous solution without soil, has as a contrast many benefits. Hydroponics have higher production per unit area, lower energy cost and are not reliant on the good soil quality. Because of these factors, the use of hydroponics has increased in recent years and is now a common practice in the greenhouse industry. *(Van Os 1999; "Dutch Greenhouse Technology | DutchGreenhouses®")*

Hydroponic systems can be either open (most commonly) or closed. In open systems, the nutrient solution is discarded at the end, which has a negative impact on the environment. In closed hydroponic systems, the nutrient solution is recycled and used again. Considering environmental aspects, the closed system is better as it reduces the use of water and prevents release of unused fertilizers in the environment and thus polluting it. However, a challenge in closed system is the spreading of root exudates that contain different compounds (organic acids…) which can inhibit growth of the plants. A method to degrade or remove these compounds must therefore be in place. *(Hosseinzadeh et al. 2017)*

A new concept, similar to closed hydroponic system is organic hydroponics (organoponics), where recycled fertilizer has the organic origin (animal manure, food waste and other organic rests) (Figure 1.8). Organic fertilizers cannot be used directly in hydroponics as they contain compounds that are detrimental to the plants. This can be solved by pre-processing of the organic fertilizer with microorganisms that convert organic compounds containing nitrogen into nitrate. Shinohara and colleagues have developed a system, where efficiency of the conversion of organic nitrogen into nitrate via ammonification and nitrification was 97.6 % and thus possible to use it for vegetable cultivation *(Shinohara et al. 2011)*.

A research shows that Rhizospheric bacteria can be beneficial for vegetable production under organoponic system *(Orberá Ratón et al. 2014)*. *Azospirillum* is one of the best characterized plant growth-promoting bacteria (PGPB). It colonizes roots of the plants and is able of nitrogen fixation, biosynthesis of plant growth hormones and promoting the root formation. For this reasons it is very beneficial for the plants and has a high potential for use in the organoponic systems. *(Steenhoudt and Vanderleyden 2000)*

Figure 1.8 *Organoponic growing of tomatoes in Nibio research greenhouse at Særheim*

1.5 GC-MS

1.5.1 Basic principles of gas chromatography

Gas chromatography (GC) is used to analyse samples in many different industries - from research, pharmaceutical, food and petroleum industry to environmental and clinical analysis. Gas chromatography coupled to mass spectrometry (GC-MS) is a powerful technique that allows separation and determination of the compounds in complex samples.

As in chromatography in general, the separation of components in the sample occurs due to different distribution between a mobile phase and a stationary phase. In GC the mobile phase is an inert gas (usually helium) and is often called a carrier gas. The stationary phase can be either a solid or a liquid. *(Skoog, Holler et al. 2007)*

GC differs from other types of chromatography because the mobile phase does not interact with the molecules of analyte, but only serves as a transport through the heated column. Components of the sample are separated mainly because of different vapour pressures, but

also because of different solubility in the stationary phase. More volatile compounds have shorter retention times (t_R) and are eluted from the column first. GC oven is usually programmed with a temperature gradient from low to high temperature in order to maximize the sample separation and minimize analysis time. Since the molecules of the analyte are carried through a column in a gas phase, the essential prerequisite for analysis with GC is that sample is volatile and thermally stable. However, when compounds of interest have too high boiling point (low volatility), as for example organic acids, amino acids and sugars, they can still be analysed by GC if they are derivatized into more volatile molecules prior to analysis. *(Skoog, Holler et al. 2007)*

1.5.2 Components of GC-MS instrumentation

Main components of the GC-MS system are shown in Figure 1.9.

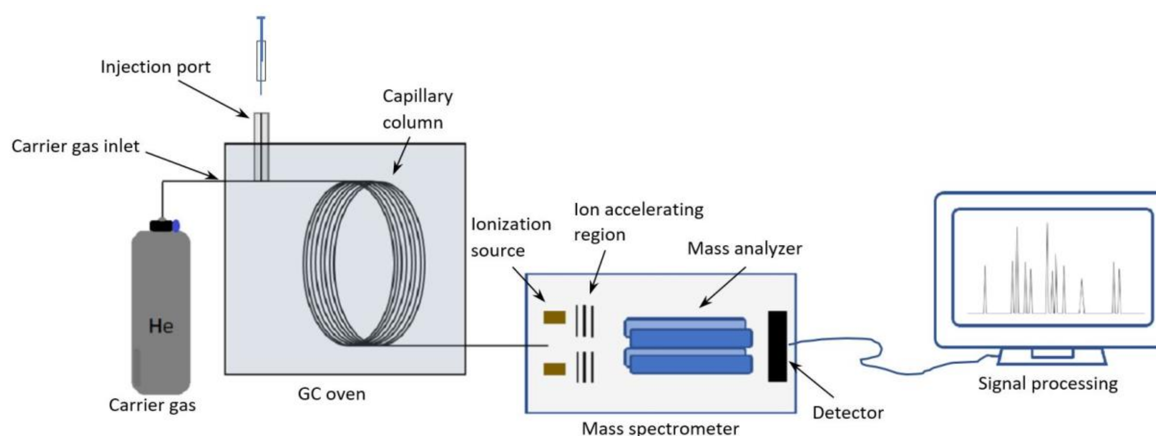

Figure 1.9 *Main components of GC-MS system. The sample is injected through the injection port, vaporized and transferred by carrier gas through the capillary column, where the separation of the analytes occurs. Components of the sample are further transferred into the inlet of mass spectrometer, where ionization, fragmentation and detection take place. Finally, the electrical signal from detector is processed and interpreted by data system.*

Carrier gas serves as mobile phase. It must be chemically inert and extremely pure (>99.999% pure). Most commonly used gas for this purpose is helium, although hydrogen and nitrogen are sometimes also used *(Dunnivant 2017)*.

The most common injector type in GC is a split-splitless injector, that can be operated in two modes. Normally, between 0.2 to 1 µL of the sample is injected into the capillary column. If

a sample contains high concentrations of analytes this volume of injected sample may lead to overloading of the column and poor separation. In this case, the split mode of injection can be used, where only a small fraction of an injected sample (*e.g.* 1:50 or 1:500) enters the column and the rest goes to waste. For samples containing low concentrations of analytes the injector can be operated in a splitless mode, where first 30 – 60 seconds are operated in a splitless mode. During this time the majority of the injected sample is loaded onto the column and after that the split mode is switched on allowing the remaining vapor being vented. *(Skoog, Holler et al. 2007; Dunnivant 2017)*

Open tubular capillary columns are today used for most of the GC applications. Capillary columns are made of fused silica glass, that is a very inert form of pure glass. Due to its fragility, fused silica is on the outer walls coated with polymer resistant to high temperatures. Stationary phase in capillary columns is a thin film of a covalently bonded liquid to the internal walls of the column and this is the place where the sample separation occurs. Capillary columns can be from 5 to 100 m long with inner diameter typically between 100 to 530 µm. *(Skoog, Holler et al.2007; Dunnivant 2017)*

GC can be coupled to different types of detectors, but the most powerful among them is mass spectrometer, that measures mass-to-charge ratio (m/z) of ions produced from the sample. Mass spectrometer is connected to a gas chromatograph by transfer line that connects the GC column outlet and the vacuum system of the mass spectrometer. Main compartments of mass spectrometer are ion source, mass analyser and detector.

Most common ion sources in GC-MS are electron ionization (EI) and chemical ionization (CI). In EI, molecules of analyte are bombarded by accelerated electrons (70 eV) which causes them to lose an electron. Primary product of this process is a molecular ion that corresponds to molecular mass of the analyte. Since analyte molecules are bombarded by highly energetic electrons, they get a large excess of energy that is lost by further fragmentation of molecules itself into a large number of single charged cations of different masses. Because of the large fragmentation this ionization technique is referred as a hard ionization technique. Since in the chamber of MS is a high vacuum ($< 10^{-5}$ Torr) molecular ions never bump into any other molecules. As a result, the EI gives a very reproducible fragmentation pattern for each molecule. This reproducibility allows us to compare and

identify compounds by similarity of their mass spectra with the spectra stored in a database library, such as NIST. *(Skoog, Holler et al. 2007)*

After gas-phase ions (fragments) are produced, they are accelerated into the mass analyser where they get separated according to their mass-to-charge ratio (m/z). The most commonly used mass analysers are quadrupoles and ion-traps. The separated fragments are detected, converted into an electrical signal (by the electron multiplier) and recorded. *(Skoog, Holler et al. 2007)*

Mass spectrometer can collect data in two modes, in full scanning mode (scan) or in selected ion monitoring mode. In scan mode, spectral data are monitored over a range of masses (mass-to-ratio values), beginning at the smallest m/z values of the fragments to the highest m/z values expected. The full scan mode provides information of the composition of the sample and is very useful for qualitative analysis*. (Bhanot 2016; Skoog, Holler et al. 2007)*

1.5.3 Derivatization

Derivatization is often a part of sample preparation procedures for gas chromatography analysis. In general, derivatization is a chemical reaction that modifies analyte molecules in a way that they become suitable for GC analysis. The requirement for GC analysis is that analytes are thermally stable and volatile at temperatures below 350 – 400 ^{O}C. If compounds do not meet these criteria, the separation can lead to nonreproducible peak areas, heights, and shapes. Therefore, these compounds need to be derivatized (chemically changed) prior to analysis to improve the resolution and peak shape (reduced tailing). *(Sellers and Corporation; "Derivatization Rgts Brochure")*

Polar compounds like organic acids, sugars and amino acids possess functional groups (-OH, -COOH, -NH, and -SH) that can form strong intermolecular forces (hydrogen bonding), which makes molecules less volatile. These polar groups in molecules need to be therefore chemically converted into a non-polar groups in order to become more volatile and suitable for GC analysis. *("Derivatization Rgts Brochure")*

Types of derivatization reactions for GC analysis are silylation, acylation, alkylation and esterification. Trimethylsilylation is most commonly applied and can be used to derivatize a wide range of molecules that contain hydroxyl, carboxyl, amino and thio groups. By displacing the active hydrogen on polar groups and transforming the molecules into sylil derivatives (Figure 1.10), they become more volatile, less polar, and thermally more stable. *("Derivatization Rgts Brochure"; Hill and Roessner 2013)*

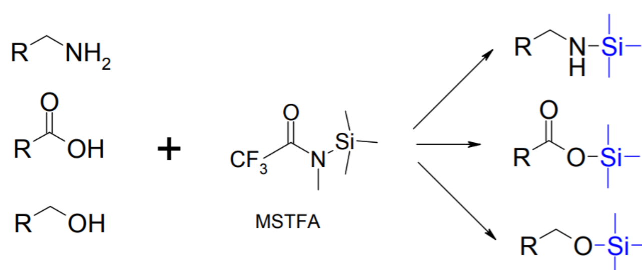

Figure 1.10 *Chemical derivatization reaction of polar molecules containing amino, carboxyl and hydroxy group by silylating reagent N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). Active hydrogen in polar functional groups is replaced with a TMS [-Si(CH3)3] group.* *(Villas-Bôas et al. 2011)*

2 MATERIALS AND METHODS

2.1 Experimental design

Two experiments were conducted. Experiment 1 was designed to investigate whether organic fertilizer with added plant growth promoting bacteria (PGPB) *Azospirillum brasilense* affects the quality and content of organic acids in tomato fruits variety Dometica. The experiment consisted of six treatments with three replicates. Light intensity was constant (176 Wm^{-2}) and applied from the top. Tomato plants in treatments 1 (NS1) and 2 (NS2) were grown in a standard nutrient solution, plants in treatments 3 (BF1) and 4 (BF2) in biorest solution (organic fertilizer) and plants in treatments 5 (BFK1) and 6 (BFK2) in the low concentration of standard nutrient solution that corresponded to concentration of nutrients in organic fertilizer. In addition, in the treatments 2, 4 and 6 PGPB *Azospirillum* were added, while treatments 1, 3 and 5 were without added bacteria. Tomato fruits were harvested three times after steady state in the plants was established. Dates of harvests were: 03.01.2019 (harvest 1), 09.01.2019 (harvest 2) and 21.01.2019 (harvest 3). Treatments in experiment 1 are listed in Table 2.1.

Table 2.1 *Experiment 1 - effects of organic fertilizer with added growth promoting bacteria on tomato fruit quality. NS indicates standard nutrient solution, BF indicates biorest nutrient solution and BFK indicates low concentration of standard nutrient solution. Treatments labelled with 1 were without growth promoting bacteria, while in treatments labelled with 2 growth promoting bacteria was added.*

Treatment	Top light intensity (Wm^{-2}) - HPS lamps	Nutrient solution	PGPB *Azospirillum*
1	176	NS1	No
2	176	NS2	Yes
3	176	BF1	No
4	176	BF2	Yes
5	176	BFK1	No
6	176	BFK2	Yes

In the experiment 2 the effect of top-light and inter-row light on the quality and content of organic acids in tomatoes variety Dometica was investigated. The experiment 2 consisted of three replicates of seven treatments with different combinations of top light intensity (High pressure sodium lamps - HPS) and inter-row light intensity (Light emitting diodes - LED). The light intensities for each treatment in experiment 2 are listed in Table 2.2. Likewise in the experiment 1, tomato fruits were harvested three times, after steady state in the plants was established. Dates of harvest 1, harvest 2 and harvest 3 were 05.12.2018, 17.12.2018 and 07.01.2019, respectively.

Table 2.2 *Experiment 2 - Effects of top-light and inter-row light on the qualities of tomatoes variety Dometica.*

Treatment	Top light intensity (Wm^{-2}) - HPS lamps	Inter-row light intensity (Wm^{-2}) - LED
1	176	0
2	176	70
3	176	140
4	263	0
5	263	70
6	300	0
7	300	70

Flow diagram of the experimental work is shown in Figure 2.1.

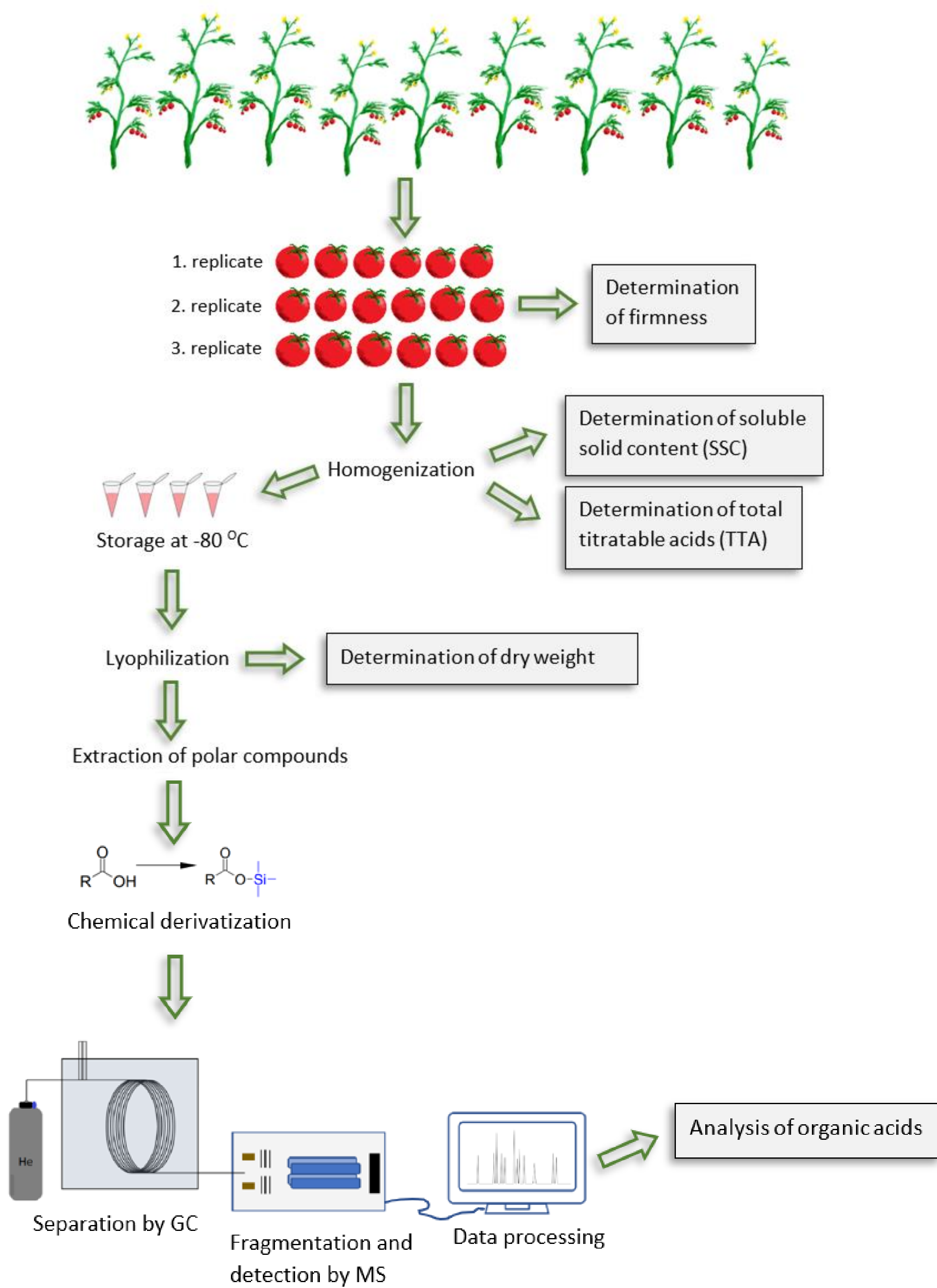

Figure 2.1 *Flow diagram of experimental work*

2.2 Plant material and growth conditions

Tomato plants (*Solanum lycopersicum*) variety Dometica were grown in a greenhouse of NIBIO research station at Særheim (Norway) in three compartments in the period from 12.09.2018 to 08.02.2019. Plants for the experiment 1 were grown on expanded clay pebbles that were previously incubated with growth promoting bacteria *Azospirillum brasilense Sp 245*, while the plants for experiment 2 were grown on a rock wool.

For the top light radiation, two types of high-pressure sodium (HPS) lamps were used: Philips GP Plus 750 (750 W) and Philips GP Plus 600 (600 W) (Gavita Nordic AS, Norway). In addition to the top light, the supplemental inter-row light was provided by led emitting diodes (LED) that combined blue (peak at the wavelength 450 nm) and red (peak at the wavelength 660 nm) light. Applied irradiation was 70 Wm^{-2} for one row of LED modules and 140 Wm^{-2} for two rows of LED modules. Measured light intensity at 10 cm distance was 58 Wm^{-2} (for one module of LED).

Conditions in the greenhouse (temperature, relative humidity, CO_2 concentration, applied irradiance, natural sun irradiation and total irradiance) are shown in Table 2.3.

***Table 2.3** Conditions in the greenhouse compartments. Data was derived from instant measurements with 5-minute interval.*

Compartment	Period	Temp. (°C)	Relative humidity (%)	CO_2 conc. (ppm)	Radiation from sun (Wm^{-2})	HPS type used (W)	Applied top light irradiation - HPS lamps (Wm^{-2})	Top light + sun irradiation (Wm^{-2})	Applied inter-row irradiation - LED (Wm^{-2})
20	12.09.2018 - 08.02.2019	21.3	68	682	30	750	176	206	Varies; 0, 70 or 140
21	12.09.2018 - 08.02.2019	22.3	75	666	30	600	273	303	Varies: 0, 70 or 140
22	04.09.2018 - 25.02.2019	22.4	74	670	33	600+750	300	333	Varies: 0 or 70

2.3 Chemicals and equipment

List of chemicals and instruments used are shown in tables 2.4 and 2.5, respectively.

Table 2.4 *List of chemicals used for quality analysis and GC-MS analysis of tomato samples*

Chemical	CAS number	Vendor	Product number
N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA)	24589-78-4	Sigma-Aldrich, Germany	394866
Methoxyamine hydrochloride (MEOX)	593-56-6	Sigma-Aldrich, Germany	226904
Pyridine	110-86-1	VWR, USA	83684230
Methanol	67-56-1	Supelco, Germany	100837
Chloroform	67-66-3	Sigma-Aldrich, Germany	25669
Ethyl acetate	141-78-6	Sigma-Aldrich, Germany	10911289736
Sodium Hydroxide	1310-73-2	Sigma-Aldrich, Germany	105590
GC-MS Standards			
Citric acid	77-92-9	Sigma-Aldrich, Germany	47264
D-Malic acid	6915-15-7	Sigma-Aldrich, Germany	47264
L-Ascorbic acid	50-81-7	Sigma-Aldrich, Germany	47264
L-Dehydroascorbic acid	490-83-5	Sigma-Aldrich, Germany	261556
Succinic acid	110-15-6	Sigma-Aldrich, Germany	47264
Shikimic acid	138-59-0	Sigma-Aldrich, Germany	47264
Quinic acid	77-95-2	Sigma-Aldrich, Germany	47264
Oxalic acid	144-62-7	Sigma-Aldrich, Germany	47264
Malonic acid	141-82-2	Sigma-Aldrich, Germany	47264
Maleic acid	110-16-7	Sigma-Aldrich, Germany	47264
Fumaric acid	110-17-8	Sigma-Aldrich, Germany	47264
Butyric acid	107-92-6	Sigma-Aldrich, Germany	47264
L-Glutamic acid	56-86-0	Sigma-Aldrich, Germany	G1251
L-Glutamine	56-85-9	Sigma-Aldrich, Germany	G3126
Ribitol (Adonitol)	488-81-3	Supelco, Germany	47266
C8-C40 alkanes calibration standard	/	Sigma-Aldrich, Germany	40147-U
Helium	7440-59-7	Praxair, USA	UN1046

Table 2.5 *List of instruments used for quality and GC-MS analysis of tomato samples.*

Instrument	Type	Vendor
Firmness tester	Durofeel	Agro-technologies, France
Refractometer	Refractometer PR-101α	Atago, Japan
Titrator	794 Basic Titrino	Metrohm, Switzerland
Freeze dryer	BK-FD10S	Biobase, China
Incubator	Incubating mini shaker	VWR, USA
Vortex mixer	Vortex-Genie 2	Scientific Industries, USA
Centrifuge	Micro Star 17R	VWR, USA
Autosampler	MPS	Gerstel, Germany
Gas chromatograph	6890 GC	Agilent Technologies, USA
Mass spectrometer	5975 MSD	Agilent Technologies, USA

2.4 Quality of the tomatoes

2.4.1 Fruit selection and sample preparation

For each light and nutrient treatment three parallel samples were harvested, where each sample (replication) consisted of six tomato fruits picked at random from different plants within one treatment. Colour of the harvested fruits was determined visually by using a scale from 1 (green) to 12 (deep red) (provided by Bama). Only tomatoes with colour corresponding to value 8 were chosen for further analysis (Figure 2.2). Firstly, firmness of each tomato fruit was measured. Thereafter, tomato fruits were divided into quarters. One quarter from each of the six tomatoes in one replication were homogenized with a blender to the uniform mixture.

One part of homogenized sample was used for quality analysis - soluble solid content (SSC) and total titratable acidity (TTA). Another part was weighed, transferred into 1.5 mL Eppendorf tubes and immediately frozen in liquid nitrogen and stored at -80 ^{o}C until further analysis with GC-MS and dry matter content determination (Figure 2.1). Measurements of firmness, SSC and TTA were performed the same day as harvesting, following the procedures published by Mitcham and co-workers *(Mitcham et al. 1996; Verheul et al. 2015)*.

Figure 2.2 *Tomatoes arranged by the colour from green (1) to deep red (12). Tomatoes with colour corresponding to value 8 were chosen for the analysis.*

2.4.2 Firmness

Firmness of the tomato fruits was measured by Durofel firmness tester (Agro-technologies, France). Each individual fruit within one replicate was punctured on three points evenly distanced from each other on the side site of the fruit pericarp. Average of all eighteen measurements in one replicate was noted and the firmness was given in scale from 1 to 100, where 100 means full firmness and 1 complete lack of firmness *(Verheul et al. 2015)*.

2.4.3 Soluble solid content

Soluble solid content was measured with a digital Refractometer PR-101α (Atago, Japan) at temperature 20 ^{o}C. A drop of a homogenized tomato sample was put on a sensor and the soluble solid content expressed as ^{o}Brix (percent of dissolved solids in a solution) was read. For a blank, distilled water was used.

2.4.4 Total titratable acidity

Total titratable acidity was measured by titration of the sample with 0.1 M NaOH, using automatic titrator 794 Basic Titrino (Metrohm, Switzerland). For each sample, approximately 5 g of a homogenized tomatoes were weighed. 100 mL of distilled water was added to the sample and titrated with NaOH to an endpoint of pH 8.2. Total titratable acidity was calculated by using Equation 2.1 *(Garner et al.)*, where milliequivalent factor for citric acid was 0.064. Total titratable acidity was expressed as percent of citric acid

equivalents (CAE) per fresh weight (FW). Instrument was calibrated with buffers at the beginning of the titration and after every third measurement.

$$\% \, CAE \; = \frac{(mls \; NaOH \; used) \cdot (0.1 \; M \; NaOH) \cdot (milliequivalent \; factor) \cdot 100}{grams \; of \; sample} \qquad \textit{Equation 2.1}$$

2.4.5 Determination of dry matter content

Dry matter content (DMC) was determined gravimetrically by drying a known amount of homogenized tomato samples in a freeze dryer (Biobase, China). When a constant weight was reached (after approximately two days of drying) the samples were weighed, and DMC was determined.

2.4.6 Statistical analysis of quality analysis

Total titratable acidity, soluble solid content and firmness of the samples were described as the average of eighteen tomatoes (3 replications x 6 tomato fruits per replicate = 18 tomato fruits per one sample) by using MS Excel. Data from each harvest was processed and analysed separately. One-way ANOVA test was performed by using SigmaPlot 14.0 software in order to find whether there were significant differences between treatments at the level of significance $P < 0.001$. For the pairwise comparison of the treatments, the Holm-Sidak method at the overall significance level $P < 0.05$ was done by using SigmaPlot 14.0.

2.5 GC-MS analysis of organic acids

2.5.1 Standards

2.5.1.1 Qualitative standards

A very small amount of each organic acid standard was transferred to separate GC vial, chemically derivatized as described in chapter 2.5.2.2 and run on a GC-MS. For each organic acid the obtained spectrum was compared to commercially available mass spectrum library NIST to confirm that the spectra corresponded to the compound of interest. Thereafter, our own reference library was built containing mass spectra for all acids with corresponding retention indexes that were determined under our conditions.

2.5.1.2 Quantitative standards

Standard dilutions (400 ppm, 200 ppm, 100 ppm, 25 ppm, 5 ppm 1 ppm and 0.5 ppm) of citric and malic acid were prepared in 50% MeOH. 20 µL of each standard dilution was transferred into a GC vial, placed for 1 hour into -80°C in order to freeze and then dried overnight under vacuum in the freeze drier. Dried standards were derivatized as described in chapter 2.5.2.2 and run on a GC-MS. After the analysis and data processing, calibration curve was made for each organic acid.

2.5.2 Sample preparation for GC-MS analysis

The extraction and derivatization was adapted from the method described in chapter 1.2 written by *(Hill and Roessner 2013)* of the book "The Handbook of Plant Metabolomics".

2.5.2.1 Extraction of polar compounds

Frozen homogenized samples stored at -80 °C were lyophilized in a freeze dryer (Biobase, China) for approximately 48 hours. Thereafter, the samples were weighed, and dry weight was determined. Approximately 20 mg of dried tomato sample was transferred into a new Eppendorf tube and 750 µL of 100% (v/v) methanol (VWR, USA) was added. Sample was

vigorously shaken with a vortex (Scientific Industries, USA) for 30 seconds. Thereafter, the sample was incubated (VWR, USA) for 15 minutes at 70 °C. After incubation, the sample was centrifuged for 8 minutes at 17000 x G at room temperature (22 °C) and the supernatant was transferred into a new Eppendorf tube. Into the tube with pellet, 750 µL of 50% (v/v) methanol and 70 µL of 3.0 mg/mL internal standard ribitol was added. Sample was again shaken with vortex for 30 seconds and later centrifuged for 8 minutes at 17000 x G at 22 °C. Supernatant was combined with the previously collected one. From the tube with collected extracts, two aliquots of 5 µL and 100 µL were transferred into a separate GC vials due to a wide range of concentrations of organic acids in tomato samples. 5 µL aliquot was later used for determination of more abundant organic acids (citric acid) whereas 100 µL aliquot was used to determine organic acids that are present in lower concentrations (glutamic acid, ascorbic acid, succinic acid…). The samples in GC vials were then put for 1 hour at -80°C in order to freeze and then dried under vacuum in the freeze dryer overnight.

2.5.2.2 Derivatization

In dried extract of the samples, 40 µL of freshly prepared activation reagent methoxyamine hydrochloride (MEOX) with concentration 20 mg/mL was added. Samples were then incubated for 90 minutes at 37 °C. After the incubation, 80 µL of derivatization reagent N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) containing 0.5 % (v/v) C8-C40 alkanes was added and the samples were incubated for 30 minutes at 37 °C. Samples were analysed by a GC-MS the same day after the derivatization.

2.5.3 GC-MS analysis

Derivatized organic and amino acids were analyzed with Agilent 6890 Gas Chromatograph coupled with Agilent 5975 Inert Mass Selective Detector (Agilent Technology, USA). The chromatographic separation was performed on a 30 m long (actual length was 28.85 m) non-polar capillary column HP-5ms (Agilent Technologies, USA) with inner diameter 0.25 mm and film thickness 0.25 µm. 1 µL of the sample was automatically injected by MPS autosampler (Gerstel, Germany) equipped with a 10- µL syringe (Gerstel, Germany) in a pulsed splitless injection mode at 230 °C. Injection pulse pressure 200 kPa was hold for 2 min (pulse time). Purge time and purge flow were set to 1.9 min and 30.0 mL/min,

respectively. Inlet pressure after the injection pulse pressure was set to 57 kPa. Helium was used as a carrier gas at the constant flow rate 1.0 mL/min, with the post run flow rate 30 mL/min. The temperature program of the analysis started isothermally at 60 ^{o}C for 1 minute, followed by the temperature increase to 325 ^{o}C by 5 ^{o}C/min ramp and final hold at 325 ^{o}C for 2 minutes. Total run time of the program was 56 min. The mass spectrometer (MS) was tuned with perfluorotributylamine (PFTBA) according to manufacturer's recommendations before the analysis. It was operated at electron ionization (EI) mode at 70 eV electron ionization energy. The transfer line temperature was set to 325 ^{o}C and the ion source temperature to 250 ^{o}C. Mass spectra were recorded at frequency 5.5 scans/s with a m/z ratio scanning range from 50 to 550 and a recording start time after 5.0 min.

2.5.4 Data processing and statistical analysis

Data acquisition was carried out by MassHunter GC-MS software (version B.07.00/Build 7.0.457.0, Agilent Technologies, USA). For the identification of the compounds internal mass spectral library was created by separately analyzing standards of acids and obtaining the mass spectra and retention index for each acid as described in chapter 2.5.1.1. Identification of compounds from the samples after deconvolution was done by comparing the fragmentation patterns and retention indexes with those from the internal library. For comparison of concentration levels of organic acids in different samples, ribitol as internal standard was added. Relative response ratios of the compounds were obtained by normalization of peak areas of the compounds by the peak area from ribitol. The principal component analysis (PCA) was performed with the relative peak areas by using software MetaboAnalyst version 4.0.

3 RESULTS

Results are divided in two sections, quality measurements of the tomatoes and analysis of organic acids with GC-MS. Each of the sections is further divided into two parts: experiment 1, where the effect of organic fertilizer with added plant growth promoting bacteria (PGPB) on quality and content of organic acids in tomato fruits was examined and experiment 2, where the effect of different light conditions on the tomato fruit quality and content of organic acids in tomatoes was investigated.

3.1 Quality of the tomatoes

3.1.1 Experiment 1

3.1.1.1 Total titratable acidity (TTA)

The effects of different nutrient solutions on total titratable acidity in tomato fruits can be clearly seen in Figure 3.1. ANOVA test (details are presented in Appendix A) showed significant differences between the treatments in all three harvests. Moreover, the trend is similar in all the harvests, with TTA being highest in tomatoes grown in mineral nutrient solutions (NS1 and NS2). Difference in TTA between treatments NS1 (without PGPB) and NS2 (PGPB added) is not significant in none of the harvests. Significant differences in TTA between tomato plants grown in biorest solution (BF1 and BF2) and low concentration of mineral nutrient solution (BFK1 and BFK2), both with and without PGPB are not very evident. In harvest 1, Holm-Sidak pairwise comparison between treatment pairs showed significant difference between treatments BF2 and BFK2, while in harvest 2 no significant difference was found. Interestingly, in the third harvest comparison between treatment pairs showed that tomatoes grown in biorest fertilizer with PGPB (BF2) had significantly higher TTA than tomatoes grown in treatments BFK2, BF1 and BFK1.

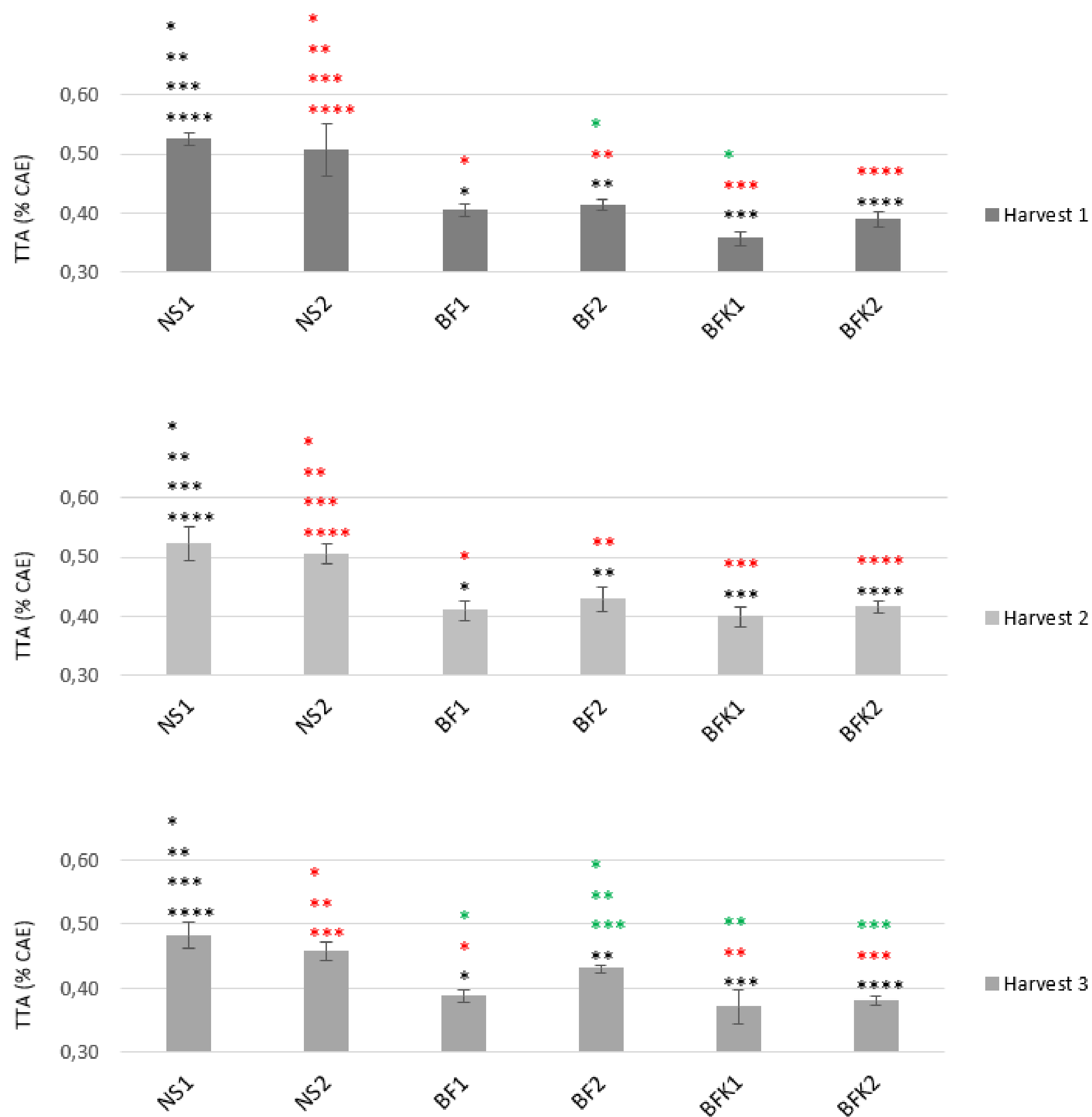

Figure 3.1 *Total titratable acidity in tomato fruits grown in different nutrient solutions expressed as percent of citric acid equivalents (% CAE). NS indicates standard nutrient solution, BF indicates biorest nutrient solution and BFK indicates low concentration of standard nutrient solution. Treatments labelled with 1 were without PGPB, while in treatments labelled with 2 PGPB was added. Asterisk signs (*) indicate treatment pairs that were significantly different. The error bars show ± standard deviation (n=3).*

3.1.1.2 Soluble solid content (SSC)

Soluble solid content (SSC) in tomato fruits is clearly affected by different nutrient solutions (Figure 3.2). By performing ANOVA test (details are shown in Appendix A) significant differences were found in all three harvests. Further pairwise comparison by Holm-Sidak method showed that tomatoes grown in mineral nutrient solutions (NS1 and NS2) had

significantly higher content of soluble solids than tomatoes where plants were grown in biorest solution (BF1 and BF2) and low concentration of mineral solution (BFK1 and BFK2).

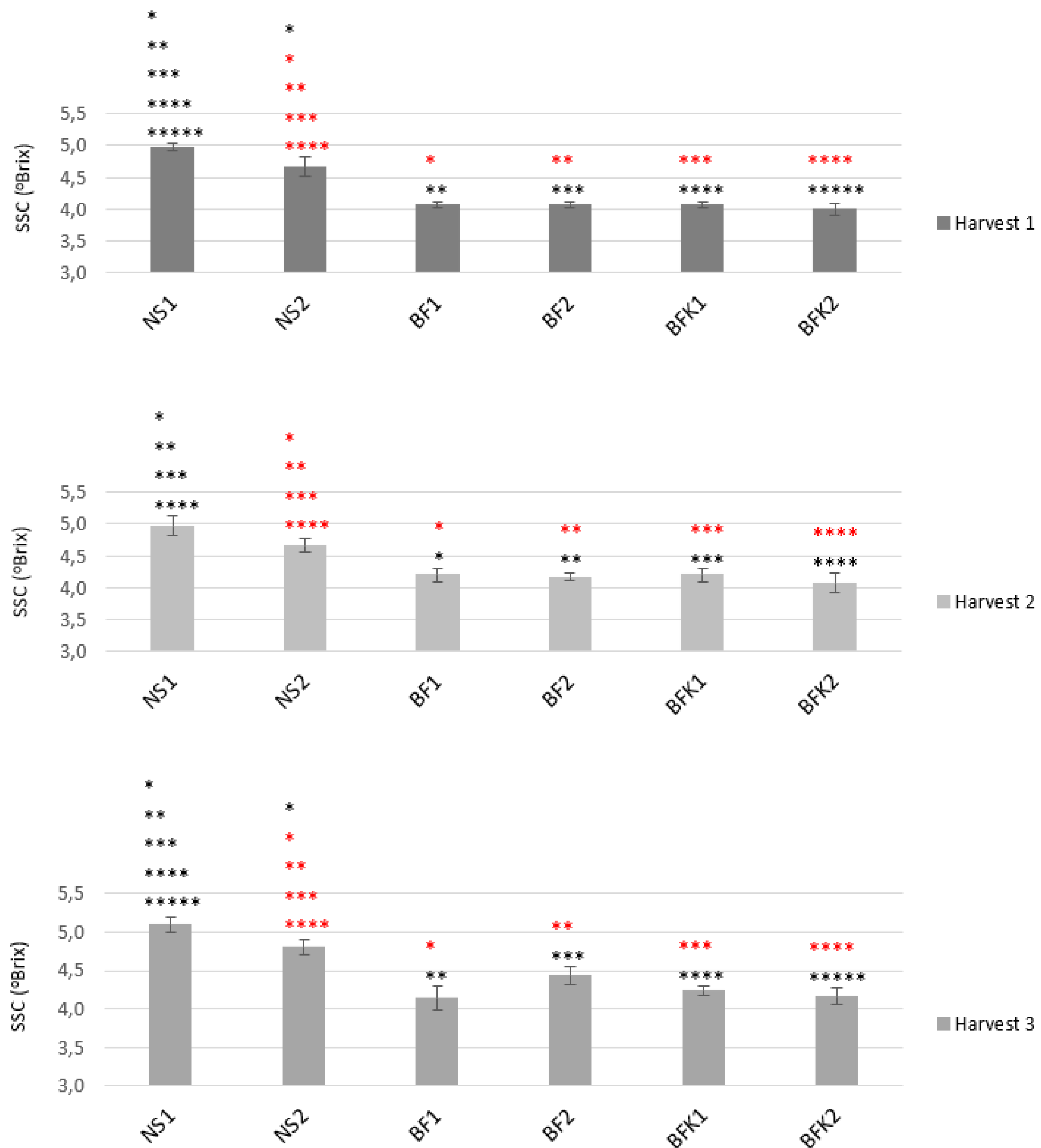

Figure 3.2 *Soluble solid content in tomato fruits grown in different nutrient solutions. NS indicates standard nutrient solution, BF indicates biorest nutrient solution and BFK indicates low concentration of standard nutrient solution. Treatments labelled with 1 were without PGPB, while in treatments labelled with 2 PGPB was added. Asterisk signs (*) indicate treatment pairs that were significantly different. The error bars show ± standard deviation (n=3).*

3.1.1.3 Firmness

Average firmness of tomato fruits in different nutrient solution treatments is shown in Figure 3.3. The results do not show very clear trend. In the first harvest, ANOVA test (details are shown in Appendix A) did not show any significant differences between the treatments, while in the second and third harvest significant differences were found. Tomatoes grown in organic fertilizer with PGPB (BF1) had significantly lower firmness as tomatoes grown in other treatments. In harvest 3, both treatments with organic fertilizer (BF1 and BF2) had a significantly lower firmness compared to other treatments.

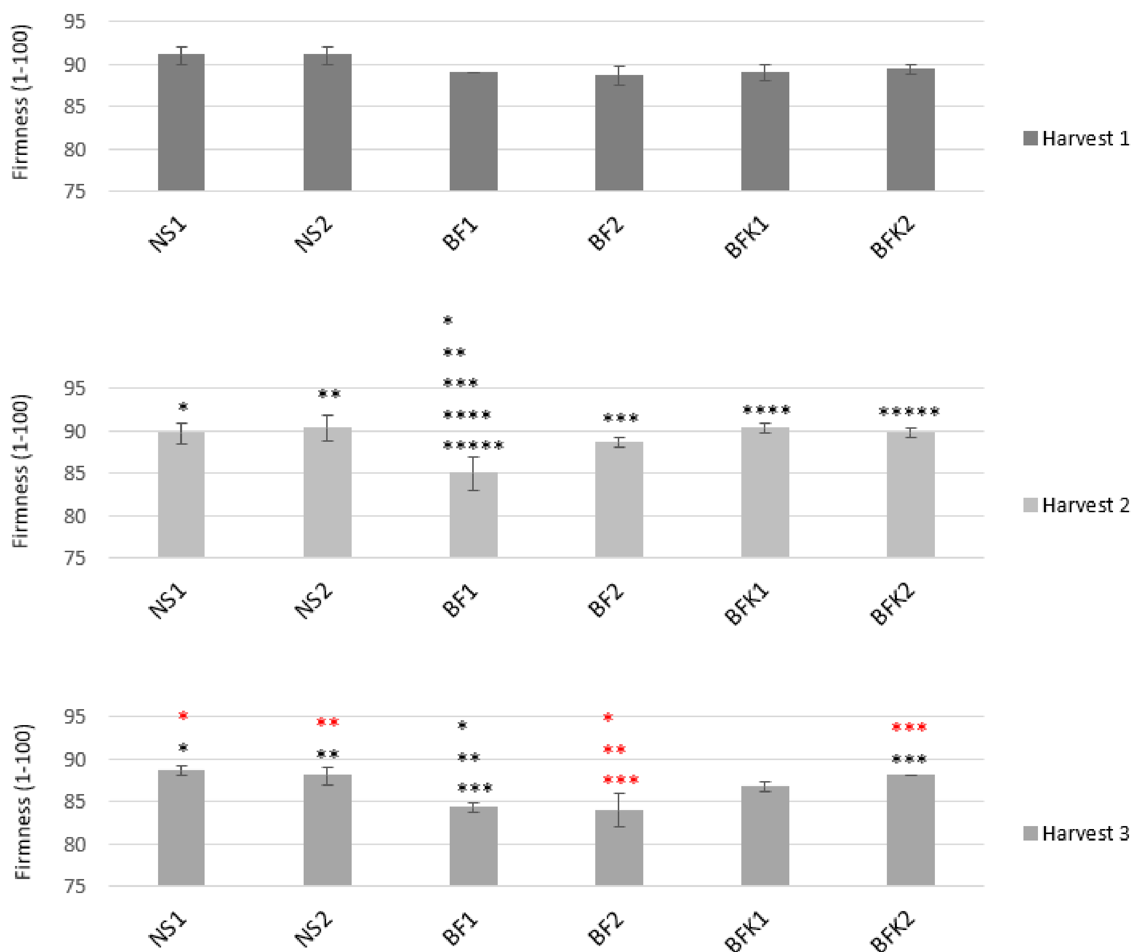

Figure 3.3 *Firmnes of tomato fruits grown in different nutrient solutions. NS indicates standard nutrient solution, BF indicates biorest nutrient solution and BFK indicates low concentration of standard nutrient solution. Treatments labelled with 1 were without PGPB, while in treatments labelled with 2 PGPB was added. Asterisk signs (*) indicate treatment pairs that were significantly different. The error bars show ± standard deviation (n=3).*

3.1.1.4 Dry matter content (DMC)

Dry matter content of tomato fruits was expressed as percentage of dry matter weight after drying relative to fresh weight of the same sample prior drying. As can be seen in Figure 3.4, DMC was highest in tomato fruits grown in standard nutrient solutions (NS1 and NS2). Detailed results of DMC are described in Appendix A (Table A3)

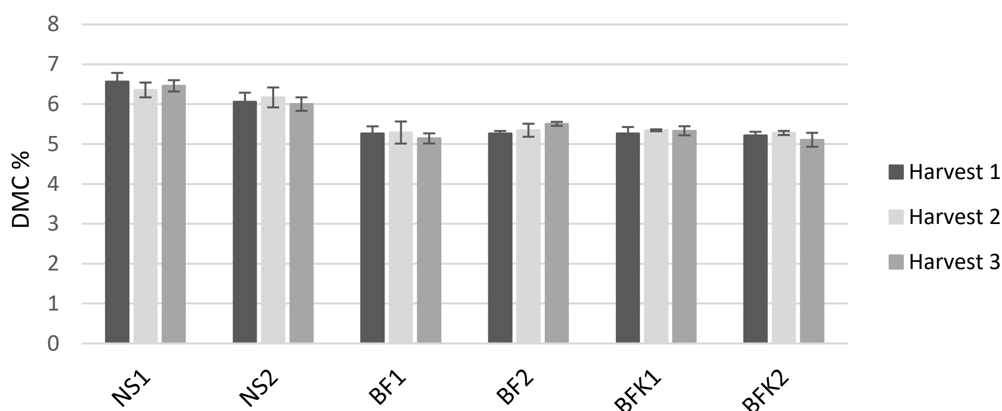

Figure 3.4 *Dry matter content of tomato fruits grown under different nutrient solutions, expressed as the percentage of dry matter after drying. The error bars show ± standard deviation (n=3).*

3.1.2 Experiment 2

3.1.2.1 Total titratable acidity (TTA)

Average levels of TTA in tomato fruits grown under different light conditions are shown in Figure 3.5. Results of ANOVA test (details are shown in Appendix B) showed no statistically significant difference in TTA between the light treatments in harvest 1 and harvest 3. However, in the second harvest, the levels of TTA in tomatoes were significantly different between the light treatments. Pairwise comparison using Holm-Sidak method showed that treatment T263_LED70 was significantly different than treatments T263_K, T300_K, T300_LED70, T176_LED70 and T176_LED140. Furthermore, treatment T176_K was significantly different than T300_K.

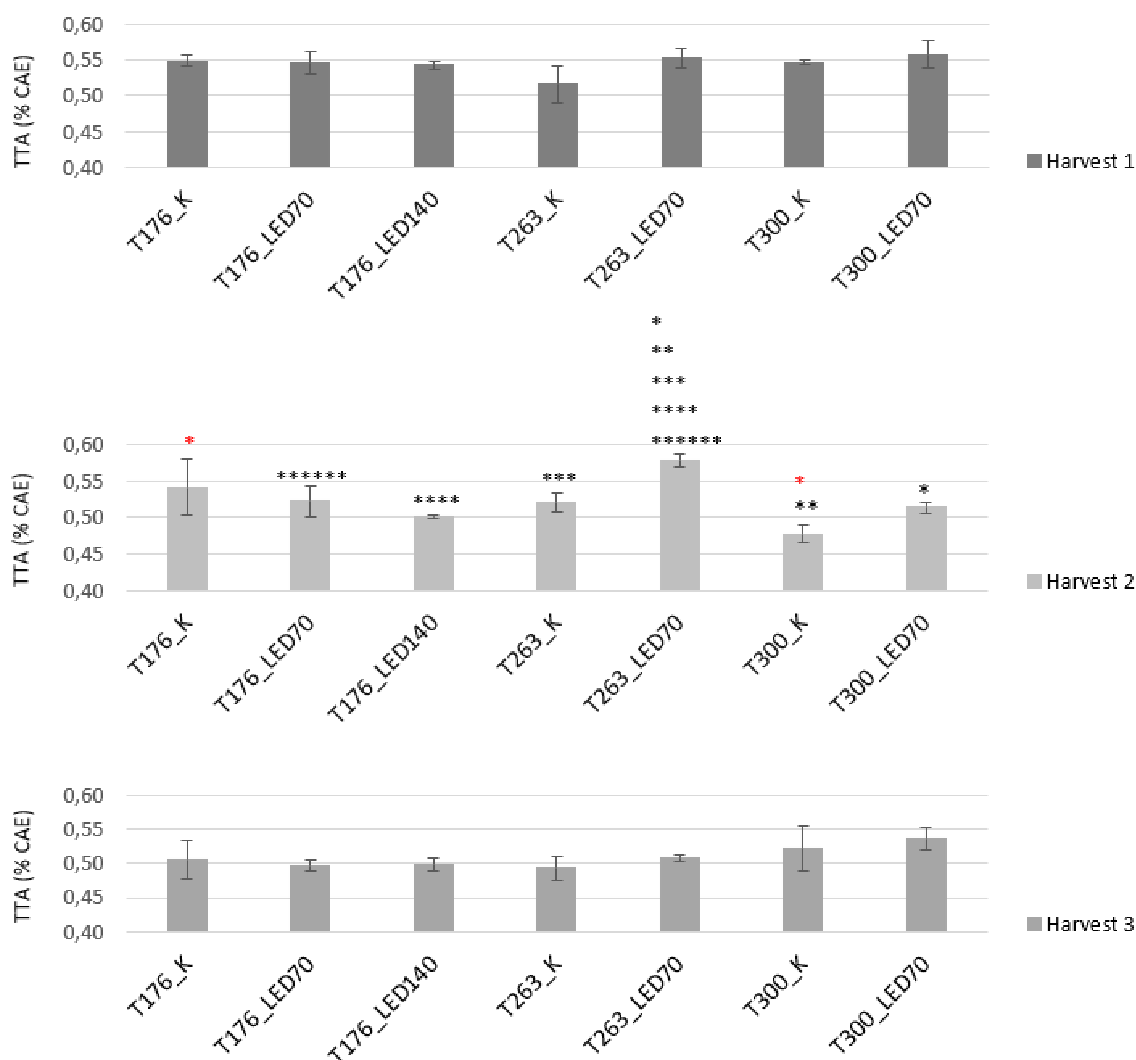

Figure 3.5 *Average levels of total titratable acidity in tomato fruits grown under seven different light conditions expressed as percent of citric acid equivalents (% CAE). T indicates top light intensity in Wm^{-2}, K indicates no inter-row lighting and LED indicates inter-row light intensity in Wm^{-2}. Pairs of treatments that were significantly different are marked with asterisk (*) signs. The error bars show ± standard deviation (n=3).*

3.1.2.2 Soluble solid content (SSC)

The content of soluble solids in tomato fruits was significantly different between light treatments in all three harvests. Detailed results from ANOVA tests are shown in Appendix B, while graphical presentation of the results is shown in Figure 3.6. In the first harvest, comparison between treatment pairs showed that SSC in tomatoes grown under top light intensity of 176 Wm^{-2} with inter-row light intensity of 140 Wm^{-2} (T176_LED140) was significantly lower than treatments where top light intensity was higher (T263_K,

T263_LED70 and T300_LED70). In harvests 2 and 3, pairwise comparison between the treatments showed even higher variation. Tomatoes that received the lowest amount of light (T176_K and T176_LED70) had significantly lower SSC than tomatoes that received higher light intensity (T176_LED140, T263_K, T263_LED70, T300_K, T300_LED70).

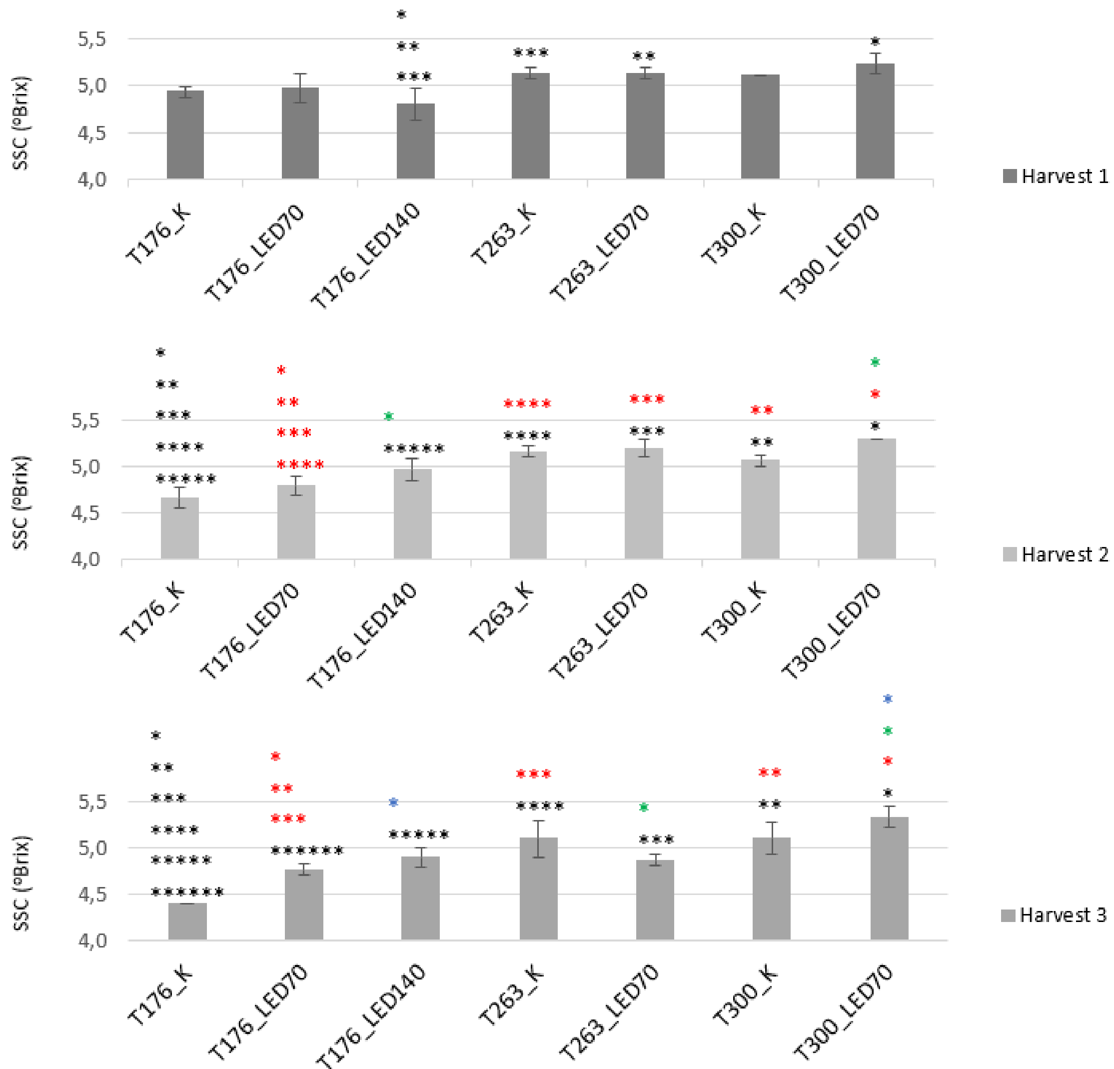

Figure 3.6 *Average SSC in tomato fruits grown under seven different light conditions. T indicates top light intensity in Wm⁻², K indicates no inter-row lighting and LED indicates inter-row light intensity in Wm⁻². Asterisk signs (*) indicate treatment pairs that were significantly different. The error bars show ± standard deviation (n=3).*

3.1.2.3 Firmness

The results from testing of firmness are shown in Figure 3.7. In all three harvests significant differences between the light treatments were found (detailed results of ANOVA tests are shown in Appendix B). It can be seen that higher light intensity, both from the top HPS lamps and the inter-row LED lighting reduced firmness of tomato fruits significantly.

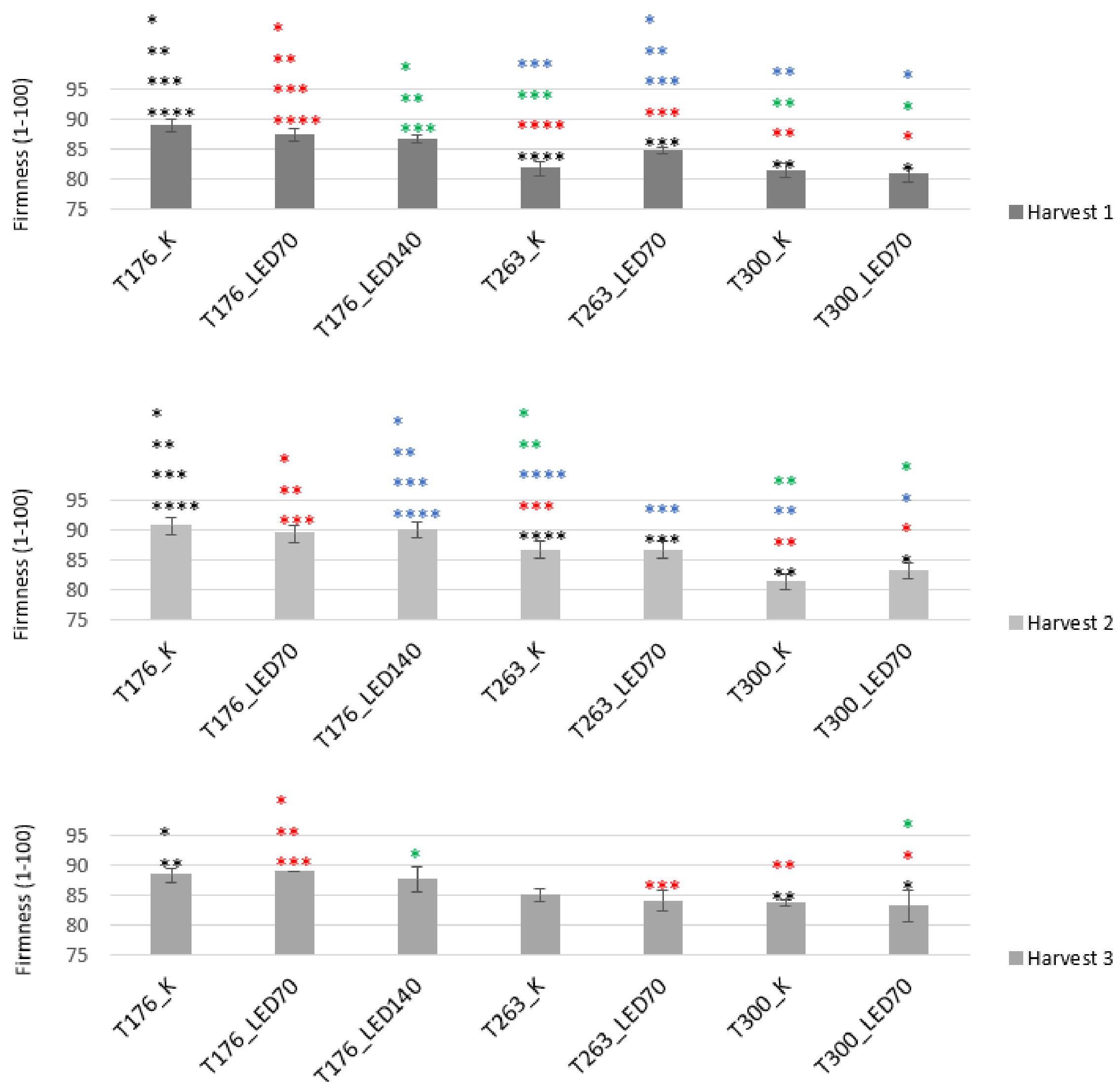

Figure 3.7 *Average firmness of tomato fruits grown under different light conditions. T indicates top light intensity in* Wm^{-2}*, K indicates no inter-row lighting and LED indicates inter-row light intensity in* Wm^{-2}*. Asterisk signs (*) indicate treatment pairs that were significantly different. The error bars show ± standard deviation (n=3).*

3.1.2.4 Dry matter content (DMC)

Dry matter content of tomato fruits was expressed as percentage of dry matter weight after drying relative to fresh weight of the same sample prior drying. DMC did not vary a lot between the samples (Figure 3.8). Sample T176_LED70 showed unexpectedly high standard deviation. Detailed results of DMC are described in Appendix B (Table B3)

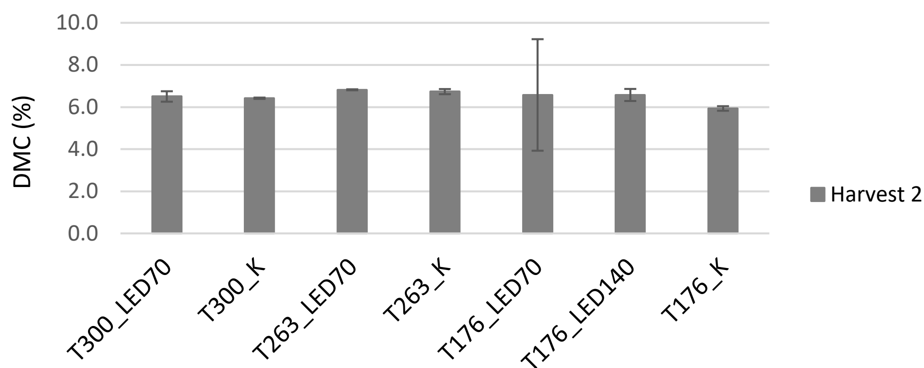

Figure 3.8 *Dry matter content of tomato fruits grown under different light treatments, expressed as the percentage of dry matter after drying. The error bars show ± standard deviation (n=3).*

3.2 GC-MS analysis of organic acids

In both experiments, tomato fruits from the second harvest were chosen for the analysis with GC-MS.

3.2.1 Experiment 1

3.2.1.1 Total ion chromatogram

Figures 3.9 and 3.10 show fragments of representative total ion chromatograms (TIC) of 5 µL and 100 µL polar phase extracts of tomato fruits analysed on GC-MS. A challenge in GC-MS analysis of tomato extracts is that citric acid (as well as some sugars) are present in high amounts, while other organic acids are present in significantly lower concentrations. To address this problem, two extracts (5 µL and 100µL) of each sample were prepared and analysed. In Figure 3.9 separated peaks of high abundant compounds (citric acid and sugars) can be seen, while compounds that are present in very small amounts are not seen. In TIC of 100 µL extract of tomato fruit (Figure 3.10) some peaks of low abundant organic acids can be seen together with overloaded peaks of citric acid and sugars.

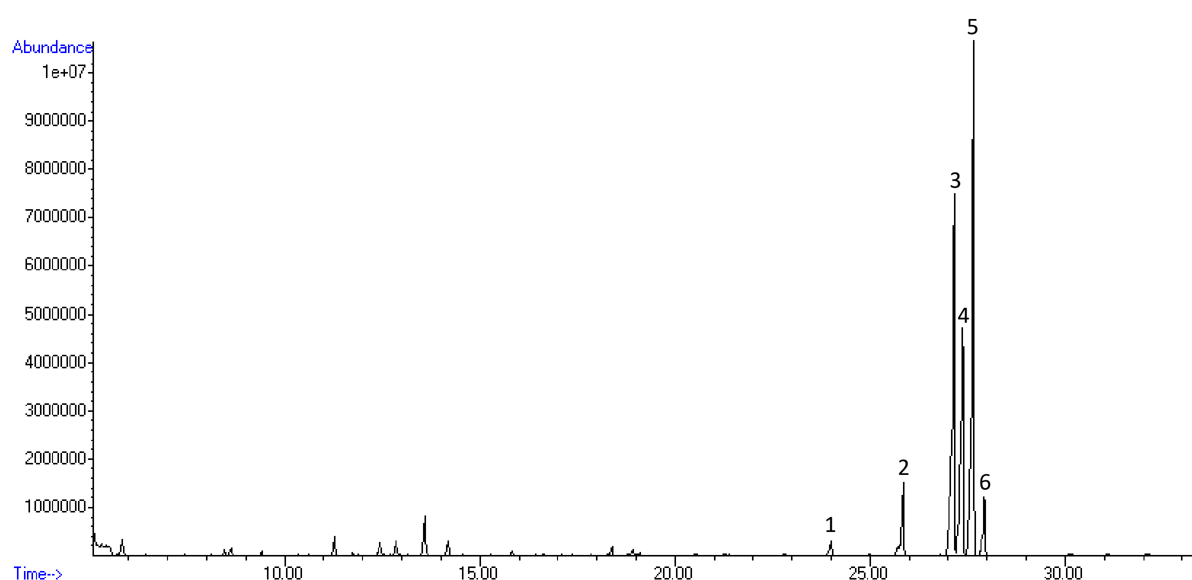

Figure 3.9 *Fragment of total ion chromatogram (TIC) of 5 µL extract of tomato fruit, sample BF1. Peak identification: 1, ribitol internal standard; 2, citric acid; 3,4,5,6 different sugars.*

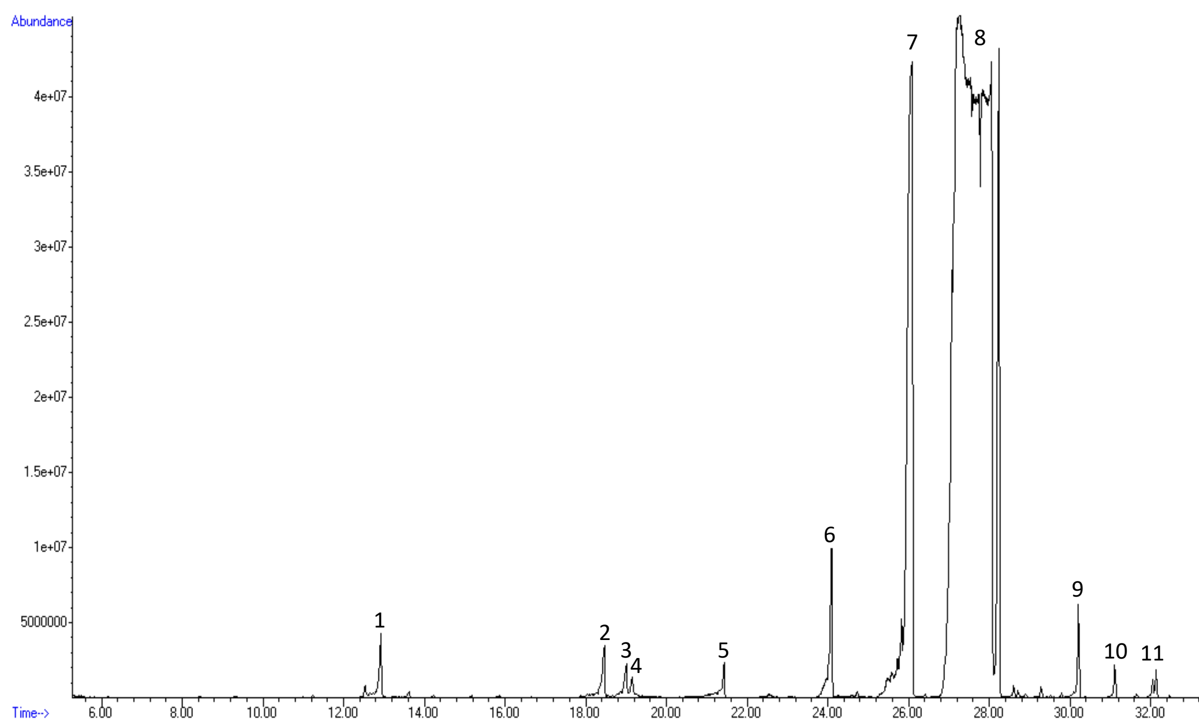

Figure 3.10 *Total ion chromatogram (TIC) of 100 µL extract of tomato fruit, sample BF1. Peak identification: 1, phosphoric acid; 2, succinic acid; 3, proline; 4, butyric acid; 5, glutamic acid; 6, ribitol internal standard; 7, citric acid; 8,9,10,11, different sugars. Peaks for citric acid and some sugars are overloaded.*

3.2.1.2 Relative levels of organic acids in tomato fruits

Relative contents of organic acids were obtained by dividing peak areas of organic acids by peak area of internal standard ribitol. Relative contents of detected organic acids in tomato fruits grown under different nutrient conditions are shown in Table 3.1 and Figure 3.11.

Table 3.1 *Relative content of organic acids in tomato fruits grown under different nutrient conditions. Response of organic acids was normalized to response for ribitol as internal standard. Data are presented as mean ± standard deviation of three replicates. GABA, gamma-aminobutyric acid; DHA, dehydroascorbic acid.*

Organic acid	NS1	NS2	BF1	BF2	BFK1	BFK2
Citric	4.40 ± 0.40	3.54 ± 0.14	1.92 ± 0.63	1.68 ± 0.45	1.41 ± 0.18	0.99 ± 0.07
Malic	0.85 ± 0.14	1.00 ± 0.17	0.82 ± 0.07	1.24 ± 0.16	1.18 ± 0.09	1.05 ± 0.14
GABA	0.74 ± 0.07	0.82 ± 0.07	0.32 ± 0.06	0.36 ± 0.03	0.46 ± 0.06	0.44 ± 0.11
Glutamic	0.25 ± 0.16	1.01 ± 0.08	0.68 ± 0.01	0.52 ± 0.24	0.69 ± 0.04	0.28 ± 0.07
Oxalic	0.13 ± 0.01	0.13 ± 0.00	0.15 ± 0.01	0.23 ± 0.02	0.17 ± 0.03	0.19 ± 0.07
Ascorbic	0.0028 ± 0.0005	0.0024 ± 0.0006	0.0033 ± 0.0005	0.0022 ± 0.0010	0.0014 ± 0.0004	0.0019 ± 0.0008
DHA	0.0042 ± 0.0003	0.0041 ± 0.0009	0.0101 ± 0.0007	0.0100 ± 0.0019	0.0057 ± 0.0016	0.0058 ± 0.0011
Fumaric	0.0012 ± 0.0001	0.0016 ± 0.0001	0.0013 ± 0.0001	0.0019 ± 0.0000	0.0017 ± 0.0002	0.0016 ± 0.0002
Maleic	0.0002 ± 0.0000	0.0003 ± 0.0000	0.0004 ± 0.0000	0.0006 ± 0.0001	0.0003 ± 0.0000	0.0003 ± 0.0000
Succinic	0.0044 ± 0.0004	0.0048 ± 0.0004	0.0030 ± 0.0001	0.0038 ± 0.0006	0.0049 ± 0.0003	0.0041 ± 0.0007

From histograms in Figure 3.11 it can be clearly seen that citric and gamma-aminobutyric acid (GABA) had highest concentrations in tomatoes grown in standard nutrient solutions (NS1 and NS2). Maleic and dehydroascorbic acid (DHA) had highest concentration in tomatoes grown in organic fertilizer (BF1 and BF2), while patterns for other acids are not so clear.

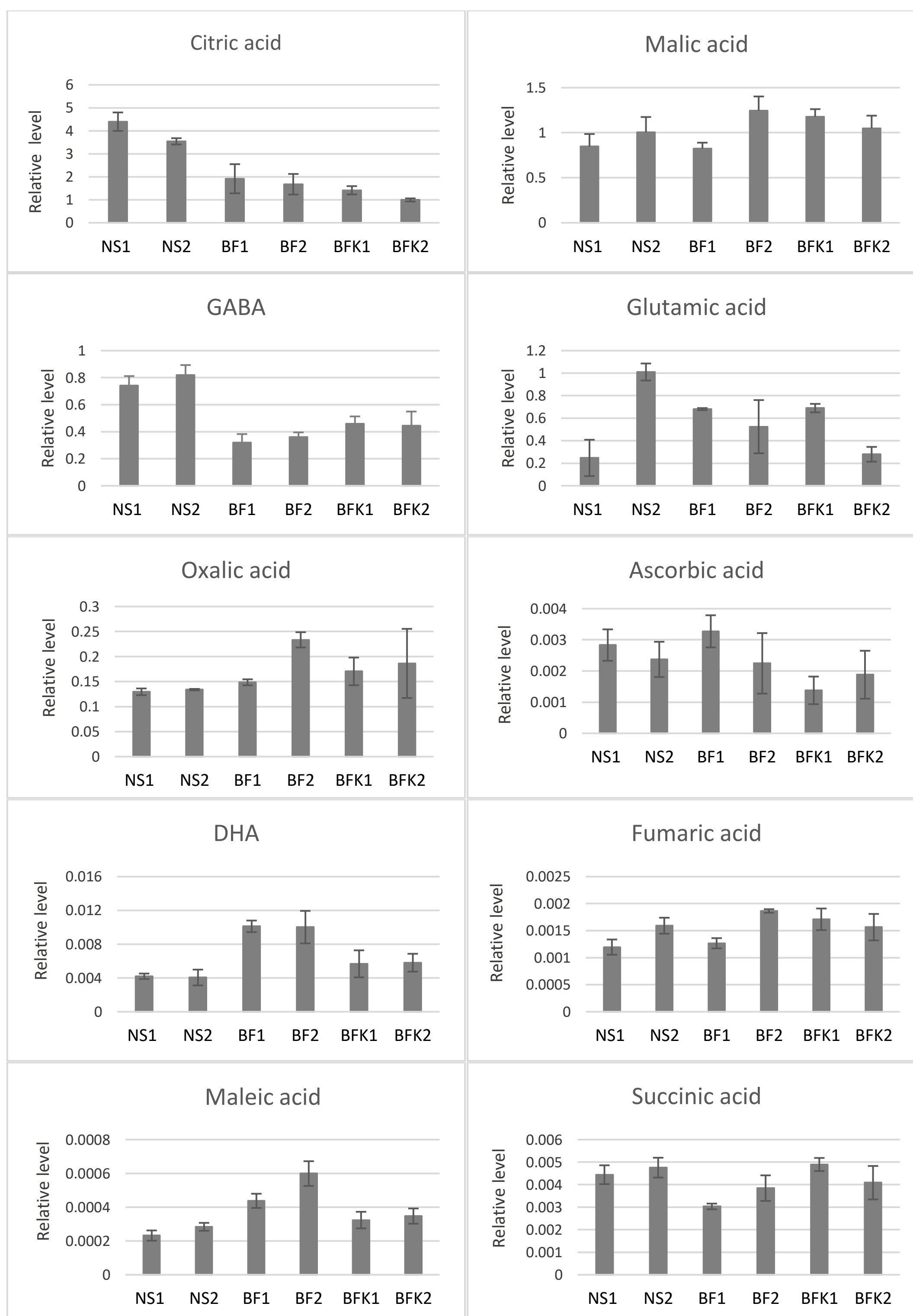

Figure 3.11 *Relative peak areas of organic acids in tomatoes grown under different nutrient conditions. The error bars indicate ± SD (n=3). GABA, gamma-aminobutyric acid; DHA, dehydroascorbic acid.*

3.2.1.3 Principal component analysis

Principal component analysis was performed in order to find differences in organic acid profiles in tomatoes grown under different nutrient conditions. First (PC1) and second principal component (PC2) captured 96.7% of the variance within the samples (Scree plot, Appendix 3, Figure C3). Score plot (Figure 3.12) shows distribution of samples on PC1 and PC2, where samples with similar organic acid profile are clustered together. It can be seen that samples from different treatments are separated along the PC1. This indicates that they have different organic acid profile. The exceptions are samples from treatments BF2 (organic fertilizer with added PGPB) and BF1 (organic fertilizer without PGPB), which are slightly spread along the x-axis. The reason for this is probably biological variation between the samples within the treatments. For samples BF1 and BF2 a separation along the y-axis (PC2) can be observed. However, PC2 accounted for only 13.1% variance which is much less than 83.5% of variance in the PC1. Attention is therefore pointed towards differences on the PC1. The loading plot (Figure 3.13) shows how strongly individual organic acid contributed to the separation on each principal component. It can be seen that citric acid is separated from other acids along the first principal component. This means that it is variation in the content of citric acid that contributes most to differences between the treatments.

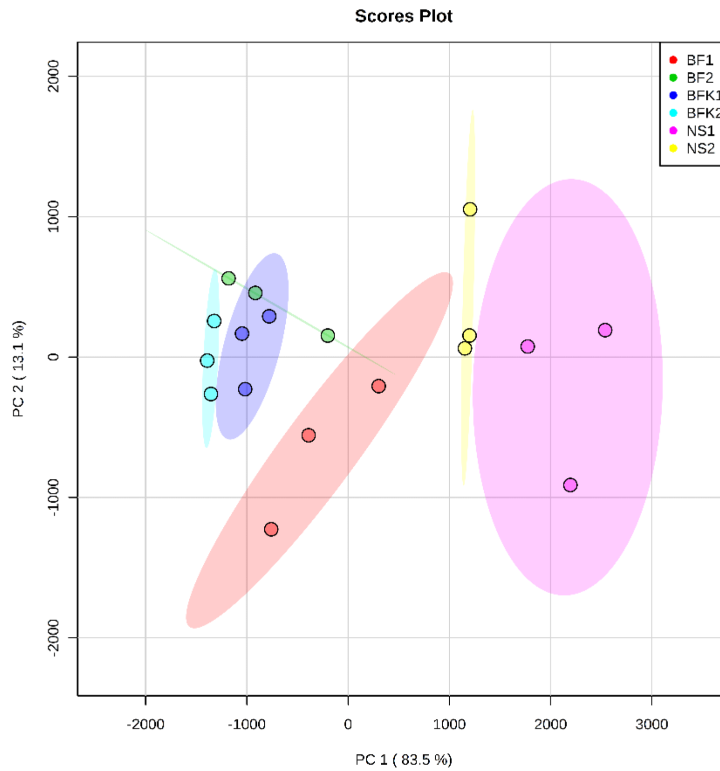

Figure 3.12 *Score plot from principal component analysis (PCA) of tomato fruits grown under different nutrient conditions. Each dot on the plot represents data from 10 organic acids in one biological replicate reduced to the first (PC1) and second principal component (PC2). The samples are colour grouped as different nutrient treatments.*

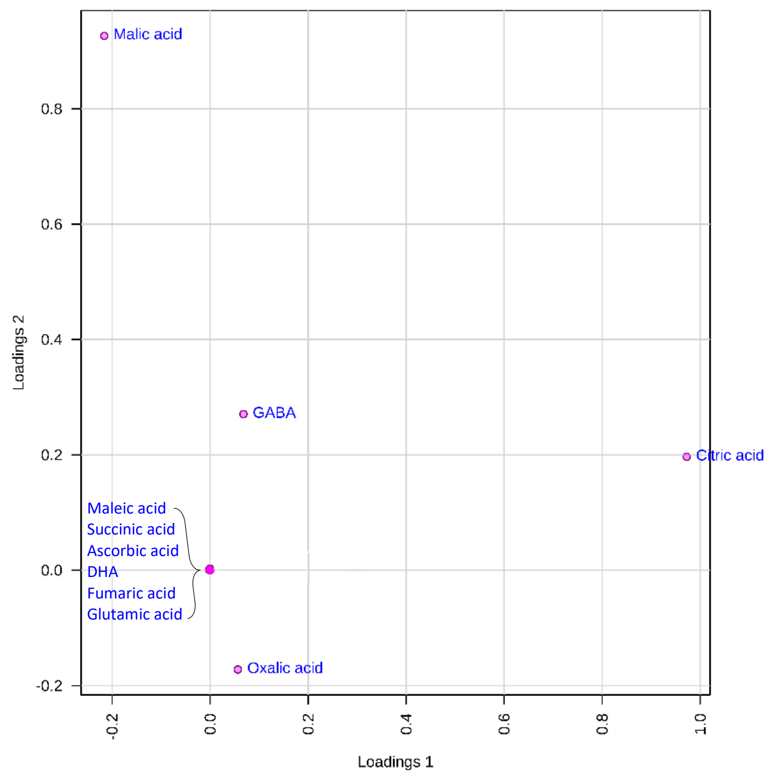

Figure 3.13 *Loading plot from PCA shows how strongly individual of organic acid influence the principal components. Citric acid has the biggest influence on the variation in the PC1. Malic acid, oxalic acid and GABA have the highest influence on the PC2. GABA, gamma-aminobutyric acid; DHA, dehydroascorbic acid.*

3.2.1.4 Absolute determination of citric and malic acid concentrations

Absolute concentrations of citric and malic acid that are known to be present in highest amounts in tomato fruits were determined by establishing calibration curves (Appendix C, Figures C1 and C2). Average concentrations of acids per dry weight of tomato fruit (mg/g) in different nutrient treatments are shown in Table 3.2. However, calibration curves were not perfectly linear, and concentrations of acids were in the lowest part of the calibration curve. These weaknesses are further discussed in Chapter 4.

Table 3.2 *Quantitative determination of citric and malic acid in tomato fruits grown under different nutrient conditions. Data are presented as average mass of organic acid per dry weight of tomato (mg/g) ± SD (n=3).*

	NS1	NS2	BF1	BF2	BFK1	BFK2
Citric acid	97 ± 21	67.2 ± 0.7	53.9 ± 7.6	45.5 ± 4.7	42.7 ± 2.6	35.1 ± 1.5
Malic acid	12.0 ± 1.1	14.7 ± 1.7	13.0 ± 2.2	20.6 ± 1.4	18.9 ± 1.0	20.0 ± 0.7

3.2.2 Experiment 2

3.2.2.1 Total ion chromatogram

Total ion chromatograms (TIC) of 5 µL and 100 µL extracts of tomato fruits grown under different light conditions are shown in Figure 3.14 and 3.15, respectively. As in experiment 1, in TIC of 5 µL extract the peaks of high abundant compounds are separated while in TIC of 100 µL extract peaks for high abundant compounds are overloaded and some of the acids with low concentration can be seen.

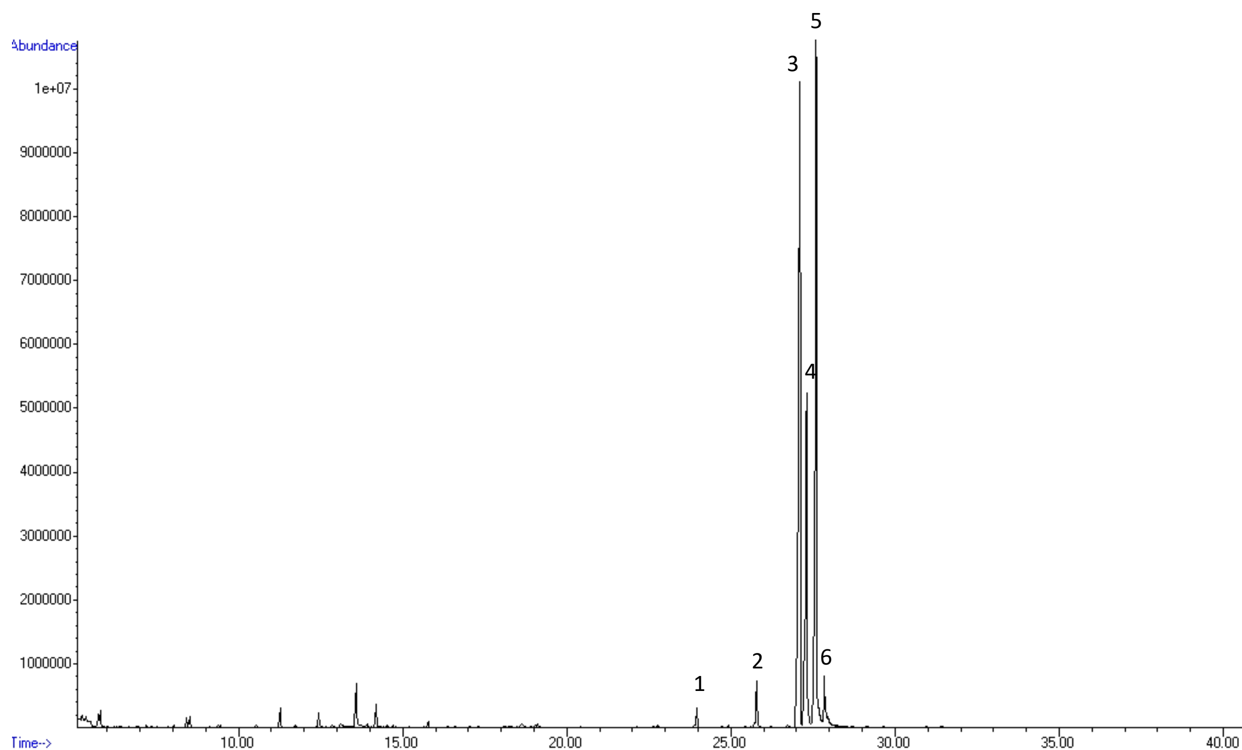

Figure 3.14 *Total ion chromatogram (TIC) of 5 µL extract of tomato fruit, sample T300_LED70. Peak identification: 1, ribitol internal standard; 2, citric acid; 3,4,5,6 different sugars.*

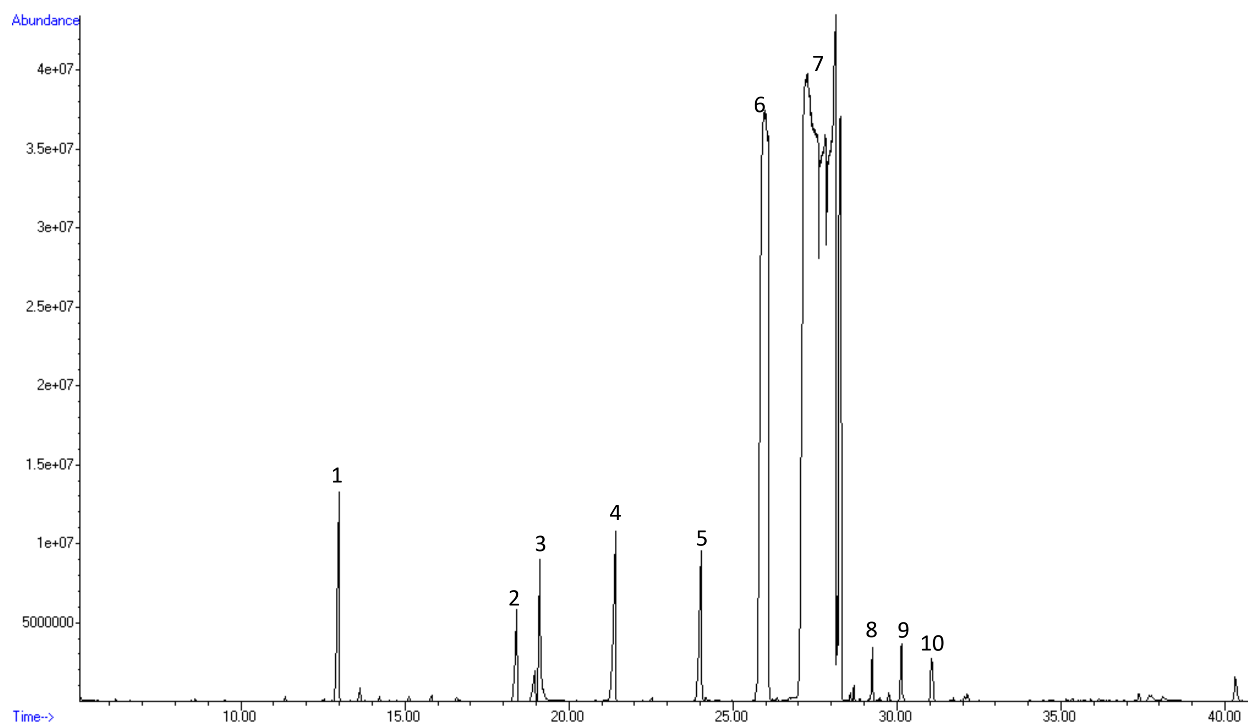

Figure 3.15 *Total ion chromatogram (TIC) of 100 µL extract of tomato fruit, sample T300_LED70. Peak identification: 1, phosphoric acid; 2, succinic acid; 3, butyric acid; 4, glutamic acid; 5, ribitol internal standard; 6, citric acid; 7,8,9,10, different sugars. Peaks for citric acid and some sugars are overloaded.*

3.2.2.2 Relative levels of organic acids in tomato fruits

Relative contents of organic acids in tomato fruits were determined based on ribitol as internal standard. Table 3.3 shows relative levels of detected organic acids in tomato fruits grown under different light conditions. Figure 3.16 shows graphical presentation of relative content of different organic acids in tomato fruits. Results for citric acid are unexpected as they show very low relative content in the treatments T176_K, T176_LED70, T176_LED140 and T263_K compared to treatments T300_K and T300_LED70, where T indicates top light intensity and LED inter-row light intensity expressed in Wm^{-2}. These results are contradictory to measurements of total titratable acidity, where so high differences between the treatments were not observed. Possible reasons for this discrepancy are discussed in Chapter 4.

Table 3.3 *Relative contents of organic acids in tomato fruits grown under different light treatments. Response of organic acids was normalized to response for ribitol as internal standard. Data are presented as mean ± standard deviation of three replicates. GABA, gamma-aminobutyric acid; DHA, dehydroascorbic acid.*

Organic acid	T176_K	T176_LED70	T176_LED140	T263_K	T263_LED70	T300_K	T300_LED70
Citric	0.03 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.08 ± 0.03	0.30 ± 0.16	0.82 ± 0.23	1.04 ± 0.49
Malic	1.14 ± 0.16	0.98 ± 0.06	0.91 ± 0.15	0.85 ± 0.03	0.94 ± 0.15	0.66 ± 0.07	0.75 ± 0.08
GABA	0.49 ± 0.06	0.57 ± 0.11	0.54 ± 0.07	0.59 ± 0.06	0.76 ± 0.10	0.71 ± 0.22	0.71 ± 0.07
Glutamic	1.45 ± 0.38	1.16 ± 0.29	1.40 ± 0.62	1.89 ± 0.31	1.86 ± 0.46	2.30 ± 0.51	2.28 ± 0.36
Oxalic	0.19 ± 0.01	0.20 ± 0.04	0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.11 ± 0.06	0.16 ± 0.01
Ascorbic	0.0014 ± 0.0006	0.0022 ± 0.0006	0.0018 ± 0.0011	0.0025 ± 0.0012	0.0031 ± 0.0010	0.0040 ± 0.0007	0.0040 ± 0.0004
DHA	0.0087 ± 0.0012	0.0072 ± 0.0014	0.0098 ± 0.0028	0.0068 ± 0.0018	0.0087 ± 0.0013	0.0091 ± 0.0028	0.0100 ± 0.0029
Fumaric	0.0036 ± 0.0030	0.0019 ± 0.0001	0.0021 ± 0.0001	0.0021 ± 0.0003	0.0018 ± 0.0001	0.0018 ± 0.0001	0.0019 ± 0.0001
Maleic	0.0007 ± 0.0005	0.0005 ± 0.0001	0.0005 ± 0.0000	0.0003 ± 0.0001	0.0004 ± 0.0000	0.0003 ± 0.0000	0.0004 ± 0.0001
Succinic	0.0071 ± 0.0046	0.0055 ± 0.0003	0.0066 ± 0.0004	0.0061 ± 0.0003	0.0078 ± 0.0010	0.0074 ± 0.0007	0.0064 ± 0.0015

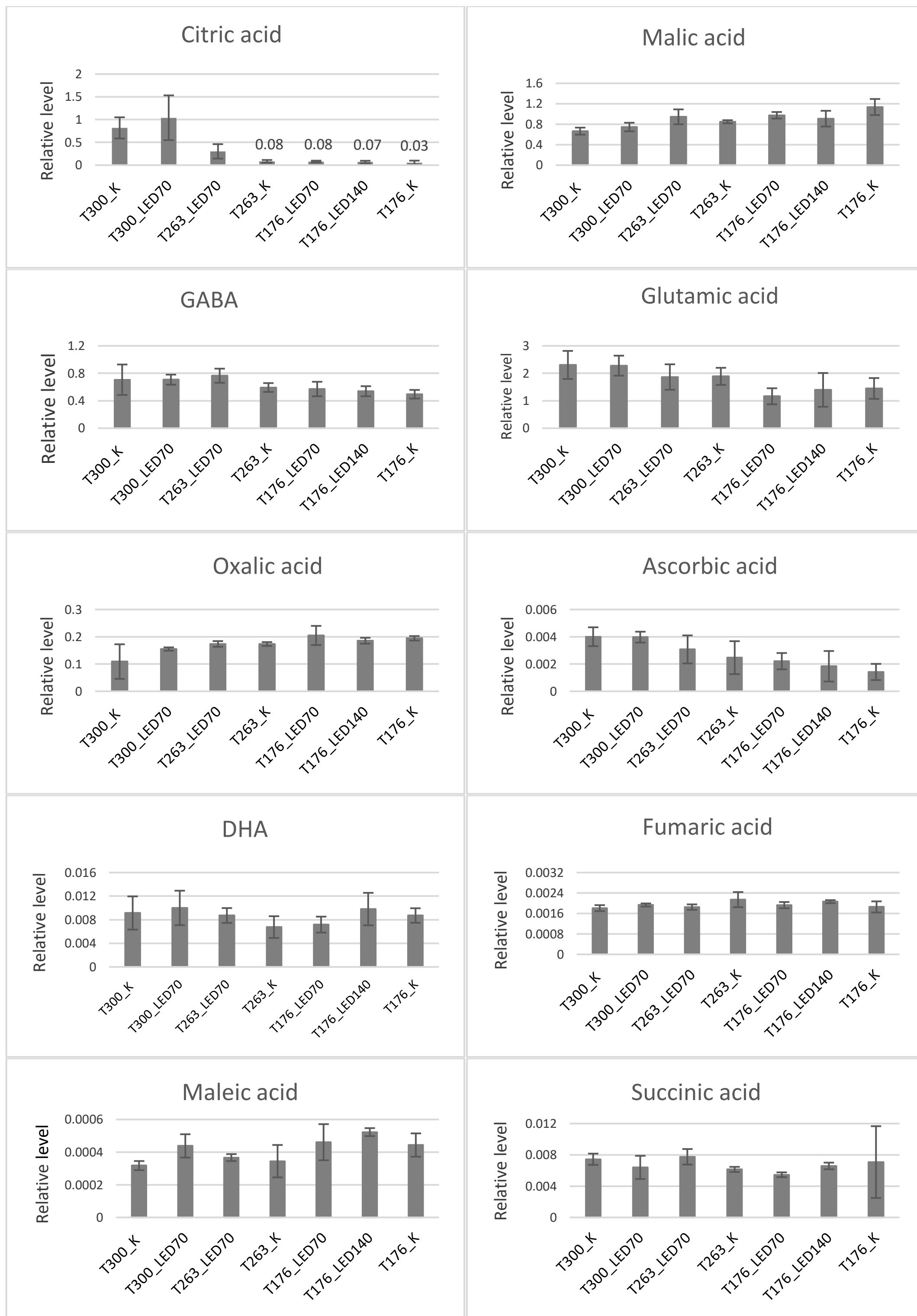

Figure 3.16 *Relative contents of organic acids in tomatoes grown under different light conditions. The error bars indicate ± SD (n=3). The smaller bars have numerical value denoted above. GABA, gamma-aminobutyric acid; DHA, dehydroascorbic acid.*

3.2.2.3 Absolute determination of citric and malic acid concentrations

Quantitative determination of citric and malic acid concentrations was done by establishing calibration curves (Appendix C, Figures C1 and C2). In Table 3.4 the average concentrations of acids per dry weight of tomato fruit (mg/g) are shown. Concentrations for malic acid are as one would expect, but the results for citric acid show unexpected low concentrations in light treatments T263_LED70, T263_K, T176_LED70, T176_LED140 and T176_K, where the concentration of citric acid is below the concentration of malic acid. This is very unlikely, as it is known that citric acid is the most abundant organic acid in tomato fruits. Possible reasons for this discrepancy are discussed in Chapter 4.

Table 3.4 *Quantitative determination of citric and malic acid in tomato fruits grown under different light conditions. Data are presented as average mass of organic acid per dry weight of tomato (mg/g) ± SD (n=3).*

	T300_K	**T300_LED70**	**T263_LED70**	**T263_K**	**T176_LED70**	**T176_LED140**	**T176_K**
Citric acid	29.2 ± 3.1	30.3 ± 6.4	13.5 ± 2.7	5.9 ± 0.8	5.7 ± 0.7	5.0 ± 0.7	4.7 ± 1.2
Malic acid	11.5 ± 0.8	12.8 ± 0.7	17.2 ± 0.9	15.0 ± 0.4	18.5 ± 1.2	16.1 ± 2.7	20.3 ± 1.7

4 DISCUSSION

4.1 Experiment 1

In experiment 1 the effect of organic fertilizer (organoponics) compared to standard mineral fertilizer as well as the effect of plant growth promoting bacteria (PGPB) *A. brasilense* on the quality of tomatoes was investigated. In addition, GC-MS analysis was performed in order to obtain more detailed profile of organic acids in tomato fruits grown under different nutrient conditions.

Quality analysis showed that tomatoes grown in standard mineral nutrient solution had significantly higher quality than tomatoes grown with organic fertilizer. Three quality characteristics were analyzed: total titratable acidity (TTA) which describes sourness, soluble solid content (SSC) that is related to content of sugars and firmness of tomato fruits. TTA was highest in tomatoes grown in mineral nutrient solution (NS1 and NS2). Differences in TTA between tomatoes grown in organic fertilizer (BF1 and BF2) and in low concentration mineral nutrient solution (BFK1 and BFK2) were not significant. The exception was in harvest 3, where TTA in treatment BF2 (organic fertilizer with added PGPB) was significantly higher than in treatments BF1 (organic fertilizer without *PGPB*), BFK1 (low concentration of mineral nutrients without PGPB) and BFK2 (low concentration of mineral nutrients with added *PGPB*). One can see a similar trend, TTA being higher in organoponic treatments where PGPB was added (BF2 and BFK2) compared to organoponic treatments without PGPB (BF1 and BFK1). In tomatoes grown in mineral nutrient solution the trend is opposite. Treatments without PGPB (NS1) had slightly higher TTA than treatments with PGPB (NS2) in all three harvests. It seems that PGPB *A. brasilense* had a positive effect on TTA in tomatoes grown in organoponic system, while in tomatoes grown in standard nutrient solution the effect was negative. Results from soluble solid content measurements showed very clearly that tomatoes grown in mineral nutrient solution had significantly higher content of sugars compared to tomatoes grown in the organoponic system. SSC between the organoponic treatments with and without PGPB (BF1, BF2, BFK1 and BFK2) did not show significant

differences. However, when comparing mineral nutrient treatments with and without PGPB, one can see that in harvests 1 and 3 significant differences were found, i.e., SSC being higher in treatments without PGPB (NS1).

The reason for different quality between the treatments could lie in different root development. Internal data from NIBIO (not presented in this thesis) showed that fresh weight of roots from mineral nutrient solution treatments (NS1 and NS2) was higher than fresh weight of roots from organic fertilizer (BF1 and BF2). This indicates that nutrient type could have an influence on the development of the root system and consequently the uptake of nutrients and thus quality of the tomato fruits.

Firmness is a characteristic that is not directly related to taste. Nevertheless, consumers associate high firmness with good quality and this trait is therefore desired. All tomatoes from our experiment had relatively high firmness. Some differences were observed in harvests 2 and 3, where tomatoes grown in the organoponic system (BF1 and BF2) had lower firmness compared to other treatments. However, the firmness in these two treatments was around 85 (on the scale from 1 to 100) which is still much higher than 40, the level that is considered non-acceptable in a Norwegian supermarket *(Verheul et al., 2015)*. Innoculation with growth promoting bacteria did not have any effect on the firmness of tomato fruits.

Analysis of organic acids in tomato fruits was performed with GC-MS, as this method is known to be highly sensitive and specific. Ten organic acids were analyzed semiquantitatively, which means that levels of acids were determined relative to the internal standard ribitol. This provides sufficient information to study differences between different treatments. In addition, for two most abundant organic acids in tomatoes, citric and malic acid, absolute concentration was determined. Concentration of citric acid was highest in treatments NS1 and NS2. This coincide well with quality analysis, where TTA was highest in NS1 and NS2, as it is well known that citric acid is the major acid in tomatoes, and it is expected that its variation will contribute most to the TTA. Highest level in treatments NS1 and NS2 had also gamma-aminobutyric acid (GABA), that has the important role in signaling and plant defense. It is known that stress (drought, heat, infection…) leads to a rapid accumulation of GABA (Bown and Shelp 2016), and increased levels in treatments NS1 and NS2 could be an indication that plants were in stress. Tomatoes grown in organoponic

system (BF1 and BF2) had highest level of dehydroascorbic acid (DHA) and one of the highest levels of ascorbic acid. These two acids are derivatives, known also as vitamin C, an antioxidant that has beneficial effects on health. Tomato fruits from organoponic treatments (BF1 and BF2) had also the highest level of maleic acid.

For other detected acids it was difficult to find any clear patterns by comparing histograms of relative levels of acids. Principal component analysis was performed in order to find which treatments were similar when comparing all ten analyzed organic acids. Results showed that treatments NS1, NS2, BFK1, BFK2 and BF1 differed from each other. Moreover, the acid that contributed most to this was citric acid. It needs to be mentioned that in some samples standard deviations were high. The most probable reason is biological variation, but human and instrumental error cannot be neglected.

Results from absolute determination of citric and malic acid were within the expected range, that is approximately 9% of dry weight for citric acid and 4% of dry weight for malic acid (Yilmaz 2001). However, the method could have been improved. The facts that calibration curves were not linear and that measured concentrations fell in the lowest part of the calibration curve are questionable. However, absolute concentrations are not of primary importance since relative data is sufficient for analysis of environmental influence on metabolite concentrations.

To summarize, quality of tomatoes was best when they were grown in standard mineral nutrient solution, and citric acid was acid that had the highest contribution to differences in total titratable acidity. Nevertheless, the quality of tomatoes grown in organoponic system was still higher as quality of imported tomatoes from Spain (internal data that is not presented in thesis). In addition, tomatoes grown in organic fertilizer (BF1 and BF2) had highest level of vitamin C, which is very important in terms of nutraceutical quality. Organoponic growing as a new concept has therefore a large potential, especially when considering the latest trends in food production where sustainability and reducing the impact on the environment is becoming more and more important.

The experiment could be repeated with precise monitoring of composition of biorest, as the lower quality of tomatoes could be caused with imbalances in nutrition provided to tomatoes. It might be also interesting to analyze more metabolites in addition to organic acids, for example sugars and amino acids.

4.2 Experiment 2

Purpose of experiment 2 was to investigate how different lighting affects the quality and contents of organic acids in tomato fruits. Two types of lighting were combined, top lights (HPS lamps) and inter-row lights (LED) that were placed next to the lower part of the plants. The idea was to provide more light to the lower leaves of the plants that would otherwise be shaded, which could lead to increased photosynthesis and higher content of sugars.

Previous research has showed that fruit quality in terms of soluble solid content and total titratable acidity was not affected by different light intensity *(Verheul 2012)*. However, in our experiment more light treatments were included.

Results of total titratable acidity (TTA) showed no statistically significant difference between light treatments in first and third harvest. In second harvest, the TTA in treatment T263_LED70 (top light intensity 263Wm^{-2}, inter-row light intensity 70 Wm^{-2}) was significantly higher than in other treatments. In addition, the TTA in treatment T176_K (top light 176 Wm^{-2}) was significantly higher than in treatment T300_K (top light 300 Wm^{-2}). These results seem to be inconclusive. When taking into account that first and second harvest did not show any significant difference between the treatments one can conclude that differences in the second harvest are due to biological variation, and that acidity of fruits was not affected by light treatments.

On the contrary, variations in soluble solid content (SSC) between different light treatments were very clear and significantly different in all three harvests. Common pattern in all three harvests was observed, i.e., higher light intensity (top light and inter-row light) led to higher SSC in tomato fruits. Particularly in harvest 2 and 3 this trend could be seen very clearly. It can be concluded that LED lighting improves quality (SSC) of tomatoes grown under low top light intensity (176 Wm^{-2}), while higher intensity of top light (263 Wm^{-2} and 300 Wm^{-2}) has even larger contribution to overall quality.

Firmness testing showed the opposite trend. Tomatoes that received more light had lower firmness than tomatoes receiving less light. However, all tomato fruits had firmness over 80 (from the scale from 1 to 100) which is considered as a very good quality.

It can be summarized that different light treatments do not affect acidity of the fruits significantly, but they do affect SSC and firmness. Another aspect, important especially for producers, is yield. In this thesis yield measurements were not included, but it would be very interesting to look into these results. In previous research it was demonstrated that higher light intensity leads to increase in the number of tomato fruits on plant, which increases the overall yield *(Verheul 2012)*. If the overall yield with LED lighting is higher and the quality of tomato fruits is not declined, it may be beneficial for tomato producers to introduce LEDs.

It might be interesting to asses quality of the tomatoes also by sensory evaluations with trained panel, which would give a direct information about tomato taste properties.

Analysis of ten organic acids in tomato fruits was performed with GC-MS. Results showed surprising values for citric acid. Levels of citric acid in treatments T300_K and T300_LED70 were much higher than in the treatments that received less light intensity (T263_LED70, T263_K, T176_LED70, T176_LED140 and T176_K). These results are contradictory to results of TTA, where in harvest 2 the highest acidity was measured in treatments T263_LED70 and T176_K. Moreover, from results of absolute determination of citric and malic acid it can be seen that concentration of citric acid in treatments T263_LED70, T263_K, T176_LED70, T176_LED140 and T176_K was lower than concentration of malic acid. This is very unlikely, and we can therefore conclude that results for citric acid are not correct. There can be several possible causes for this discrepancy. The most obvious mistake could be wrong peak identification of citric acid, which would result in a wrong peak area and thus wrong relative response. However, the spectra and data processing were reviewed, and this reason was therefore excluded. Another reason could lie in a mistake in sample preparation, but this would most probably lead to irregular results for all acids. In our experiment, the absolute results for malic acid seem to be as one would expect and therefore it is difficult to identify a reason for perplexing results of citric acid. In order to get more reliable results for content of citric acid in tomato fruits it would be best to repeat the experiment.

Nevertheless, results for content of malic acid show that the lowest concentration was in treatments with the highest light intensity (T300_K and T300_LED70) and the highest concentration in the treatment with the lowest light intensity (T176_K). Centeno and his colleagues showed that tomatoes with low levels of malate had higher levels of transitory

starch and SSC, and the opposite *(Centeno et al. 2011)*. When looking into our results of SSC one can see that the highest levels of SSC was in treatment T300_LED70 and the lowest in treatment T176_K. This corresponds well with findings of the mentioned study, where they propose that malate has a regulatory function in fruit metabolism. Higher light intensity seem to increase the contents of glutamic acid and ascorbic acid, but not the oxidized form of ascorbic acid (DHA). For other organic acids it is difficult to make any conclusions from the relative levels of individual acids. For more comprehensive analysis of organic acids the experiment should be repeated and PCA with correct results of citric acid should be performed.

5 CONCLUSION

Content of organic acids in tomato fruits is more affected by different nutrients than by different light treatments.

Quality of tomatoes was affected by both light and nutrients. Tomatoes grown in standard mineral nutrient solution had higher quality (described in terms of TTA, SSC and firmness) than tomatoes grown with organic fertilizer (organoponic system). However, organic tomatoes were better than tomatoes imported from Spain. Moreover, tomatoes grown with organic fertilizer had the highest levels of vitamin C, which is an important antioxidant and has beneficial effects on health. The organoponic system is a new concept of growing tomatoes in greenhouses that has potential for improvements. It is a promising method, especially when considering the latest trends among consumers and food producers, where reducing the negative impact on the environment and improved sustainability have become an important subject.

Different combinations of top light and inter-row LED lighting had an impact on SSC and firmness of tomato fruits, but not on the acidity. LED lighting had a positive effect on the content of sugars (SSC) in tomatoes grown under lower top light intensities. However, the effect of top light intensity had an even larger effect on the SSC and thus quality. It may be beneficial for tomato producers to introduce LED supplementary lighting in addition to high top light intensities, as it leads to higher SSC and possibly also to higher yield.

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Appendix A

Quality measurements – experiment 1

Table A1 *The measurements of total titratable acidity (TTA) in tomatoes grown under different nutrient treatments at NIBIO, Særheim in autumn 2018 / winter 2019*

Treatment	Replicate	Mass of tomato (g)	pH initial	pH final	V NaOH (mL)	% TTA	Average % TTA	SD	CV %
Harvest 1									
NS1	1	4.992	4.51	8.20	4.188	0.5369	0.525	0.010	2.0
NS1	2	4.995	4.48	8.20	4.034	0.5169			
NS1	3	5.006	4.65	8.20	4.084	0.5221			
NS2	1	5.011	4.38	8.20	3.856	0.4925	0.506	0.044	8.7
NS2	2	4.997	4.39	8.21	4.338	0.5556			
NS2	3	4.938	4.33	8.21	3.632	0.4707			
BF1	1	5.009	4.46	8.20	3.080	0.3935	0.405	0.010	2.6
BF1	2	4.965	4.42	8.20	3.156	0.4068			
BF1	3	5.038	4.56	8.21	3.258	0.4139			
BFK1	1	4.993	4.59	8.20	2.886	0.3699	0.358	0.012	3.4
BFK1	2	5.001	4.56	8.21	2.700	0.3455			
BFK1	3	5.008	4.75	8.20	2.796	0.3573			
BF2	1	5.012	4.50	8.20	3.302	0.4216	0.414	0.009	2.1
BF2	2	4.970	4.40	8.20	3.220	0.4146			
BF2	3	5.001	4.63	8.20	3.160	0.4044			
BFK2	1	5.018	4.52	8.20	3.180	0.4056	0.389	0.014	3.6
BFK2	2	5.000	4.52	8.20	2.996	0.3835			
BFK2	3	5.007	4.71	8.20	2.968	0.3794			
Harvest 2									
NS1	1	5.003	4.47	8.21	4.282	0.5478	0.522	0.029	5.5
NS1	2	5.008	4.46	8.20	4.128	0.5275			
NS1	3	4.955	4.37	8.21	3.802	0.4911			
NS2	1	4.996	4.60	8.21	3.800	0.4868	0.505	0.017	3.4
NS2	2	4.973	4.53	8.20	3.940	0.5071			
NS2	3	4.988	4.20	8.20	4.060	0.5209			
BF1	1	5.031	4.36	8.20	3.296	0.4193	0.410	0.017	4.1
BF1	2	5.003	4.36	8.20	3.276	0.4191			
BF1	3	4.975	4.26	8.21	3.034	0.3903			
BFK1	1	5.003	4.56	8.20	3.042	0.3891	0.399	0.018	4.5
BFK1	2	5.000	4.55	8.20	3.276	0.4193			
BFK1	3	5.004	4.19	8.20	3.034	0.3880			
BF2	1	4.979	4.43	8.21	3.462	0.4450	0.428	0.020	4.7
BF2	2	5.007	4.35	8.21	3.398	0.4343			
BF2	3	5.014	4.39	8.20	3.180	0.4059			
BFK2	1	4.957	4.50	8.20	3.150	0.4067	0.415	0.011	2.6
BFK2	2	4.977	4.43	8.20	3.320	0.4269			
BFK2	3	5.020	4.29	8.20	3.218	0.4103			

Treatment	Replicate	Mass of tomato (g)	pH initial	pH final	V NaOH (mL)	% TTA	Average % TTA	SD	CV %
Harvest 3									
NS1	1	4.984	5.35	8.21	3.666	0.4708	0.482	0.021	4.4
NS1	2	4.995	5.35	8.21	3.664	0.4695			
NS1	3	5.015	4.97	8.20	3.974	0.5072			
NS2	1	4.983	5.37	8.21	3.526	0.4529	0.457	0.015	3.3
NS2	2	5.000	5.34	8.21	3.704	0.4741			
NS2	3	5.055	4.99	8.21	3.516	0.4452			
BF1	1	4.996	5.24	8.20	3.034	0.3887	0.387	0.010	2.6
BF1	2	5.036	5.25	8.20	3.120	0.3965			
BF1	3	4.944	5.12	8.21	2.910	0.3767			
BFK1	1	5.031	5.35	8.21	3.044	0.3872	0.370	0.026	7.1
BFK1	2	4.960	5.35	8.21	2.972	0.3835			
BFK1	3	5.053	4.89	8.21	2.682	0.3397			
BF2	1	4.993	5.25	8.24	3.310	0.4243	0.430	0.006	1.4
BF2	2	5.033	4.91	8.25	3.434	0.4367			
BF2	3	4.995	4.75	8.20	3.360	0.4305			
BFK2	1	4.969	5.31	8.21	2.890	0.3722	0.380	0.007	1.9
BFK2	2	4.991	5.29	8.20	3.018	0.3870			
BFK2	3	5.040	4.94	8.21	2.996	0.3804			0.0

***Table A2** The measurements of firmness and soluble solid content (SSC) in tomatoes grown under different nutrient treatments at Nibio, Særheim in autumn 2018 / winter 2019*

Treatment	Replicate	Firmness	Average Firmness	SD	CV %	SSC (ºBrix)	Average SSC (ºBrix)	SD	CV %
Harvest 1									
NS1	1	91	91	1.000	1.1	4.9	4.97	0.058	1.2
NS1	2	90				5.0			
NS1	3	92				5.0			
NS2	1	91	91	1.000	1.1	4.7	4.67	0.153	3.3
NS2	2	90				4.8			
NS2	3	92				4.5			
BF1	1	89	89	0.000	0.0	4.0	4.07	0.058	1.4
BF1	2	89				4.1			
BF1	3	89				4.1			
BFK1	1	88	89	1.000	1.1	4.0	4.07	0.058	1.4
BFK1	2	89				4.1			
BFK1	3	90				4.1			
BF2	1	88	89	1.155	1.3	4.1	4.07	0.058	1.4
BF2	2	88				4.0			
BF2	3	90				4.1			
BFK2	1	89	89	0.577	0.6	4.1	4.00	0.100	2.5
BFK2	2	89				3.9			
BFK2	3	90				4.0			

Treatment	Replicate	Firmness	Average Firmness	SD	CV %	SSC (°Brix)	Average SSC (°Brix)	SD	CV %
Harvest 2									
NS1	1	89	90	1.155	1.3	5.1	4.97	0.153	3.1
NS1	2	91				5.0			
NS1	3	89				4.8			
NS2	1	90	90	1.528	1.7	4.8	4.67	0.115	2.5
NS2	2	92				4.6			
NS2	3	89				4.6			
BF1	1	87	85	2.000	2.4	4.2	4.20	0.100	2.4
BF1	2	83				4.1			
BF1	3	85				4.3			
BFK1	1	91	90	0.577	0.6	4.3	4.20	0.100	2.4
BFK1	2	90				4.1			
BFK1	3	90				4.2			
BF2	1	89	89	0.577	0.7	4.2	4.17	0.058	1.4
BF2	2	89				4.1			
BF2	3	88				4.2			
BFK2	1	90	90	0.577	0.6	4.2	4.07	0.153	3.8
BFK2	2	90				4.1			
BFK2	3	89				3.9			
Harvest 3									
NS1	1	89	89	0.577	0.7	5.2	5.10	0.100	2.0
NS1	2	88				5.0			
NS1	3	89				5.1			
NS2	1	87	88	1.000	1.1	4.8	4.80	0.100	2.1
NS2	2	88				4.9			
NS2	3	89				4.7			
BF1	1	85	84	0.577	0.7	4.1	4.13	0.153	3.7
BF1	2	84				4.0			
BF1	3	84				4.3			
BFK1	1	87	87	0.577	0.7	4.2	4.23	0.058	1.4
BFK1	2	87				4.3			
BFK1	3	86				4.2			
BF2	1	86	84	2.000	2.4	4.3	4.43	0.115	2.6
BF2	2	82				4.5			
BF2	3	84				4.5			
BFK2	1	88	88	0.000	0.0	4.1	4.17	0.115	2.8
BFK2	2	88				4.3			
BFK2	3	88				4.1			

Table A3 *Dry matter content (DMC) in tomatoes grown under different nutrient solutions at Nibio, Særheim in autumn 2018 / winter 2019*

Treatment	Replicate	DMC (%)	Average DMC (%)	SD	CV (%)
Harvest 1					
NS1	1	6.63	6.56	0.22	3.4
NS1	2	6.74			
NS1	3	6.31			
NS2	1	6.15	6.06	0.23	3.8
NS2	2	6.23			
NS2	3	5.80			
BF1	1	5.15	5.27	0.18	3.3
BF1	2	5.19			
BF1	3	5.47			
BF2	1	5.28	5.26	0.07	1.3
BF2	2	5.32			
BF2	3	5.19			
BFK1	1	5.08	5.26	0.16	3.1
BFK1	2	5.30			
BFK1	3	5.41			
BFK2	1	5.28	5.21	0.09	1.8
BFK2	2	5.11			
BFK2	3	5.25			
Harvest 2					
NS1	1	6.49	6.36	0.19	2.9
NS1	2	6.44			
NS1	3	6.14			
NS2	1	6.23	6.17	0.25	4.0
NS2	2	6.38			
NS2	3	5.89			
BF1	1	5.05	5.29	0.28	5.2
BF1	2	5.22			
BF1	3	5.59			
BF2	1	5.53	5.35	0.16	3.1
BF2	2	5.27			
BF2	3	5.24			
BFK1	1	5.37	5.35	0.02	0.5
BFK1	2	5.35			
BFK1	3	5.32			
BFK2	1	5.27	5.28	0.05	1.0
BFK2	2	5.23			
BFK2	3	5.33			

Treatment	Replicate	DMC (%)	Average DMC (%)	SD	CV (%)
Harvest 3					
NS1	1	6.55	6.46	0.14	2.2
NS1	2	6.30			
NS1	3	6.53			
NS2	1	6.08	6.00	0.17	2.8
NS2	2	6.12			
NS2	3	5.81			
BF1	1	5.19	5.14	0.13	2.5
BF1	2	5.00			
BF1	3	5.23			
BF2	1	5.55	5.51	0.05	0.9
BF2	2	5.45			
BF2	3	5.52			
BFK1	1	5.42	5.33	0.11	2.1
BFK1	2	5.38			
BFK1	3	5.20			
BFK2	1	5.31	5.11	0.18	3.4
BFK2	2	5.02			
BFK2	3	4.99			

ANOVA analysis – experiment 1

Experiment 1, Total titratable acidity, Harvest 1

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, TTA, H1

Normality Test (Shapiro-Wilk): Passed (P = 0.077)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.234)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	0.525	0.0104	0.00599
NS2	6	3	0.506	0.0441	0.0255
BF1	6	3	0.405	0.0104	0.00598
BFK1	6	3	0.358	0.0122	0.00704
BF2	6	3	0.414	0.00865	0.00499
BFK2	6	3	0.390	0.0141	0.00814

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.0679	0.0136	31.584	<0.001
Residual	12	0.00516	0.000430		
Total	17	0.0731			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BFK1	0.168	9.904	<0.001	Yes
NS2 vs. BFK1	0.149	8.780	<0.001	Yes
NS1 vs. BFK2	0.136	8.019	<0.001	Yes
NS1 vs. BF1	0.121	7.119	<0.001	Yes
NS2 vs. BFK2	0.117	6.895	<0.001	Yes
NS1 vs. BF2	0.112	6.599	<0.001	Yes
NS2 vs. BF1	0.102	5.995	<0.001	Yes
NS2 vs. BF2	0.0927	5.476	0.001	Yes
BF2 vs. BFK1	0.0560	3.305	0.043	Yes
BF1 vs. BFK1	0.0472	2.785	0.095	No
BFK2 vs. BFK1	0.0319	1.886	0.354	No
BF2 vs. BFK2	0.0240	1.419	0.551	No
NS1 vs. NS2	0.0190	1.124	0.631	No
BF1 vs. BFK2	0.0152	0.899	0.623	No
BF2 vs. BF1	0.00880	0.520	0.613	No

Experiment 1, Total titratable acidity, Harvest 2

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, TTA, H2

Normality Test (Shapiro-Wilk): Passed (P = 0.727)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.852)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	0.522	0.0287	0.0166
NS2	6	3	0.505	0.0172	0.00990
BF1	6	3	0.410	0.0167	0.00963
BFK1	6	3	0.399	0.0178	0.0103
BF2	6	3	0.428	0.0202	0.0117
BFK2	6	3	0.415	0.0108	0.00622

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.0424	0.00847	22.710	<0.001
Residual	12	0.00448	0.000373		
Total	17	0.0468			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BFK1	0.123	7.821	<0.001	Yes
NS1 vs. BF1	0.113	7.139	<0.001	Yes
NS1 vs. BFK2	0.107	6.817	<0.001	Yes
NS2 vs. BFK1	0.106	6.731	<0.001	Yes
NS2 vs. BF1	0.0954	6.048	<0.001	Yes
NS1 vs. BF2	0.0937	5.944	<0.001	Yes
NS2 vs. BFK2	0.0903	5.727	<0.001	Yes
NS2 vs. BF2	0.0765	4.854	0.003	Yes
BF2 vs. BFK1	0.0296	1.877	0.463	No
BF2 vs. BF1	0.0188	1.194	0.830	No
NS1 vs. NS2	0.0172	1.091	0.828	No
BFK2 vs. BFK1	0.0158	1.004	0.805	No
BF2 vs. BFK2	0.0138	0.873	0.784	No
BF1 vs. BFK1	0.0108	0.683	0.758	No
BFK2 vs. BF1	0.00507	0.321	0.754	No

Experiment 1, Total titratable acidity, Harvest 3

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, TTA, H3

Normality Test (Shapiro-Wilk): Passed (P = 0.907)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.849)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	0.482	0.0214	0.0124
NS2	6	3	0.457	0.0150	0.00864
BF1	6	3	0.387	0.00997	0.00576
BFK1	6	3	0.370	0.0264	0.0153
BF2	6	3	0.431	0.00620	0.00358
BFK2	6	3	0.380	0.00741	0.00428

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.0317	0.00633	24.161	<0.001
Residual	12	0.00315	0.000262		
Total	17	0.0348			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BFK1	0.112	8.500	<0.001	Yes
NS1 vs. BFK2	0.103	7.763	<0.001	Yes
NS1 vs. BF1	0.0952	7.201	<0.001	Yes
NS2 vs. BFK1	0.0873	6.601	<0.001	Yes
NS2 vs. BFK2	0.0775	5.865	<0.001	Yes
NS2 vs. BF1	0.0701	5.303	0.002	Yes
BF2 vs. BFK1	0.0604	4.566	0.006	Yes
NS1 vs. BF2	0.0520	3.933	0.016	Yes
BF2 vs. BFK2	0.0506	3.830	0.017	Yes
BF2 vs. BF1	0.0432	3.268	0.040	Yes
NS2 vs. BF2	0.0269	2.035	0.284	No
NS1 vs. NS2	0.0251	1.899	0.290	No
BF1 vs. BFK1	0.0172	1.299	0.523	No
BFK2 vs. BFK1	0.00973	0.736	0.725	No
BF1 vs. BFK2	0.00743	0.562	0.584	No

Experiment 1, Soluble solid content, Harvest 1

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, SSC, H1

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.345)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	4.967	0.0577	0.0333
NS2	6	3	4.667	0.153	0.0882
BF1	6	3	4.067	0.0577	0.0333
BFK1	6	3	4.067	0.0577	0.0333
BF2	6	3	4.067	0.0577	0.0333
BFK2	6	3	4.000	0.1000	0.0577

Source of Variation	DF	SS	MS	F	P
Between Groups	5	2.496	0.499	64.186	<0.001
Residual	12	0.0933	0.00778		
Total	17	2.589			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BFK2	0.967	13.424	<0.001	Yes
NS1 vs. BF2	0.900	12.499	<0.001	Yes
NS1 vs. BFK1	0.900	12.499	<0.001	Yes
NS1 vs. BF1	0.900	12.499	<0.001	Yes
NS2 vs. BFK2	0.667	9.258	<0.001	Yes
NS2 vs. BF1	0.600	8.332	<0.001	Yes
NS2 vs. BFK1	0.600	8.332	<0.001	Yes
NS2 vs. BF2	0.600	8.332	<0.001	Yes
NS1 vs. NS2	0.300	4.166	0.009	Yes
BFK1 vs. BFK2	0.0667	0.926	0.939	No
BF2 vs. BFK2	0.0667	0.926	0.903	No
BF1 vs. BFK2	0.0667	0.926	0.845	No
BF1 vs. BFK1	0.000	0.000	1.000	No
BFK1 vs. BF2	0.000	0.000	1.000	No
BF1 vs. BF2	0.000	0.000	1.000	No

Experiment 1, Soluble solid content, Harvest 2

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, SSC, H2

Normality Test (Shapiro-Wilk): Passed (P = 0.178)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.350)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	4.967	0.153	0.0882
NS2	6	3	4.667	0.115	0.0667
BF1	6	3	4.200	0.1000	0.0577
BFK1	6	3	4.200	0.1000	0.0577
BF2	6	3	4.167	0.0577	0.0333
BFK2	6	3	4.067	0.153	0.0882

Source of Variation	DF	SS	MS	F	P
Between Groups	5	1.904	0.381	27.424	<0.001
Residual	12	0.167	0.0139		
Total	17	2.071			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BFK2	0.900	9.353	<0.001	Yes
NS1 vs. BF2	0.800	8.314	<0.001	Yes
NS1 vs. BFK1	0.767	7.967	<0.001	Yes
NS1 vs. BF1	0.767	7.967	<0.001	Yes
NS2 vs. BFK2	0.600	6.235	<0.001	Yes
NS2 vs. BF2	0.500	5.196	0.002	Yes
NS2 vs. BF1	0.467	4.850	0.004	Yes
NS2 vs. BFK1	0.467	4.850	0.003	Yes
NS1 vs. NS2	0.300	3.118	0.061	No
BF1 vs. BFK2	0.133	1.386	0.720	No
BFK1 vs. BFK2	0.133	1.386	0.654	No
BF2 vs. BFK2	0.100	1.039	0.785	No
BF1 vs. BF2	0.0333	0.346	0.981	No
BFK1 vs. BF2	0.0333	0.346	0.930	No
BF1 vs. BFK1	0.000	0.000	1.000	No

Experiment 1, Soluble solid content, Harvest 3

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, SSC, H3

Normality Test (Shapiro-Wilk): Passed (P = 0.375)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.125)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
NS1	6	3	5.100	0.1000	0.0577
NS2	6	3	4.800	0.1000	0.0577
BF1	6	3	4.133	0.153	0.0882
BFK1	6	3	4.233	0.0577	0.0333
BF2	6	3	4.433	0.115	0.0667
BFK2	6	3	4.167	0.115	0.0667

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	5	2.304	0.461	37.709	<0.001
Residual	12	0.147	0.0122		
Total	17	2.451			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
NS1 vs. BF1	0.967	10.709	<0.001	Yes
NS1 vs. BFK2	0.933	10.340	<0.001	Yes
NS1 vs. BFK1	0.867	9.601	<0.001	Yes
NS1 vs. BF2	0.667	7.385	<0.001	Yes
NS2 vs. BF1	0.667	7.385	<0.001	Yes
NS2 vs. BFK2	0.633	7.016	<0.001	Yes
NS2 vs. BFK1	0.567	6.278	<0.001	Yes
NS2 vs. BF2	0.367	4.062	0.013	Yes
NS1 vs. NS2	0.300	3.323	0.042	Yes
BF2 vs. BF1	0.300	3.323	0.036	Yes
BF2 vs. BFK2	0.267	2.954	0.059	No
BF2 vs. BFK1	0.200	2.216	0.174	No
BFK1 vs. BF1	0.100	1.108	0.642	No
BFK1 vs. BFK2	0.0667	0.739	0.724	No
BFK2 vs. BF1	0.0333	0.369	0.718	No

Experiment 1, Firmness, Harvest 1

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, Firmness, H1

Normality Test (Shapiro-Wilk): Passed (P = 0.094)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	91.000	1.000	0.577
NS2	6	3	91.000	1.000	0.577
BF1	6	3	89.000	0.000	0.000
BFK1	6	3	89.000	1.000	0.577
BF2	6	3	88.667	1.155	0.667
BFK2	6	3	89.333	0.577	0.333

Source of Variation	DF	SS	MS	F	P
Between Groups	5	16.667	3.333	4.286	0.018
Residual	12	9.333	0.778		
Total	17	26.000			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.018).

Power of performed test with alpha = 0.050: 0.708

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BF2	2.333	3.240	0.101	No
NS2 vs. BF2	2.333	3.240	0.095	No
NS2 vs. BFK1	2.000	2.777	0.197	No
NS2 vs. BF1	2.000	2.777	0.183	No
NS1 vs. BF1	2.000	2.777	0.169	No
NS1 vs. BFK1	2.000	2.777	0.155	No
NS2 vs. BFK2	1.667	2.315	0.302	No
NS1 vs. BFK2	1.667	2.315	0.273	No
BFK2 vs. BF2	0.667	0.926	0.962	No
BFK2 vs. BFK1	0.333	0.463	0.998	No
BFK2 vs. BF1	0.333	0.463	0.995	No
BF1 vs. BF2	0.333	0.463	0.985	No
BFK1 vs. BF2	0.333	0.463	0.958	No
NS1 vs. NS2	0.000	0.000	1.000	No
BF1 vs. BFK1	0.000	0.000	1.000	No

Experiment 1, Firmness, Harvest 2

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, Firmness, H2

Normality Test (Shapiro-Wilk): Passed (P = 0.645)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.134)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	89.667	1.155	0.667
NS2	6	3	90.333	1.528	0.882
BF1	6	3	85.000	2.000	1.155
BFK1	6	3	90.333	0.577	0.333
BF2	6	3	88.667	0.577	0.333
BFK2	6	3	89.667	0.577	0.333

Source of Variation	DF	SS	MS	F	P
Between Groups	5	61.611	12.322	8.531	0.001
Residual	12	17.333	1.444		
Total	17	78.944			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.001).

Power of performed test with alpha = 0.050: 0.979

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS2 vs. BF1	5.333	5.435	0.002	Yes
BFK1 vs. BF1	5.333	5.435	0.002	Yes
BFK2 vs. BF1	4.667	4.756	0.006	Yes
NS1 vs. BF1	4.667	4.756	0.006	Yes
BF2 vs. BF1	3.667	3.737	0.031	Yes
BFK1 vs. BF2	1.667	1.698	0.706	No
NS2 vs. BF2	1.667	1.698	0.668	No
BFK2 vs. BF2	1.000	1.019	0.959	No
NS1 vs. BF2	1.000	1.019	0.938	No
NS2 vs. BFK2	0.667	0.679	0.986	No
BFK1 vs. BFK2	0.667	0.679	0.972	No
BFK1 vs. NS1	0.667	0.679	0.942	No
NS2 vs. NS1	0.667	0.679	0.882	No
NS1 vs. BFK2	0.000	0.000	1.000	No
NS2 vs. BFK1	0.000	0.000	1.000	No

Experiment 1, Firmness, Harvest 3

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 1, Firmness, H3

Normality Test (Shapiro-Wilk): Passed (P = 0.309)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
NS1	6	3	88.667	0.577	0.333
NS2	6	3	88.000	1.000	0.577
BF1	6	3	84.333	0.577	0.333
BFK1	6	3	86.667	0.577	0.333
BF2	6	3	84.000	2.000	1.155
BFK2	6	3	88.000	0.000	0.000

Source of Variation	DF	SS	MS	F	P
Between Groups	5	60.278	12.056	12.056	<0.001
Residual	12	12.000	1.000		
Total	17	72.278			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.999

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
NS1 vs. BF2	4.667	5.715	0.001	Yes
NS1 vs. BF1	4.333	5.307	0.003	Yes
NS2 vs. BF2	4.000	4.899	0.005	Yes
BFK2 vs. BF2	4.000	4.899	0.004	Yes
NS2 vs. BF1	3.667	4.491	0.008	Yes
BFK2 vs. BF1	3.667	4.491	0.007	Yes
BFK1 vs. BF2	2.667	3.266	0.059	No
BFK1 vs. BF1	2.333	2.858	0.110	No
NS1 vs. BFK1	2.000	2.449	0.196	No
BFK2 vs. BFK1	1.333	1.633	0.562	No
NS2 vs. BFK1	1.333	1.633	0.497	No
NS1 vs. NS2	0.667	0.816	0.895	No
NS1 vs. BFK2	0.667	0.816	0.815	No
BF1 vs. BF2	0.333	0.408	0.904	No
NS2 vs. BFK2	0.000	0.000	1.000	No

Appendix B

Quality measurements – experiment 2

Table B1 *The measurements of total titratable acidity (TTA) in tomatoes grown under different light treatments at Nibio, Særheim in autumn 2018 / winter 2019*

Treatment	Replicate	Mass of tomato (g)	pH initial	pH final	V NaOH (mL)	% TTA	Average % TTA	SD	CV %
Harvest 1									
T300_LED70	1	5.004	4.33	8.20	4.532	0.580	0.558	0.019	3.4
T300_LED70	2	5.000	4.38	8.20	4.260	0.545			
T300_LED70	3	5.003	4.35	8.21	4.294	0.549			
T300_K	1	4.965	4.46	8.22	4.266	0.550	0.546	0.003	0.6
T300_K	2	5.019	4.47	8.21	4.282	0.546			
T300_K	3	5.023	4.43	8.20	4.266	0.544			
T263_LED70	1	5.013	4.43	8.20	4.242	0.542	0.552	0.013	2.3
T263_LED70	2	5.069	4.49	8.20	4.344	0.549			
T263_LED70	3	5.018	4.48	8.20	4.442	0.567			
T263_K	1	5.002	4.47	8.21	3.980	0.509	0.516	0.026	5.1
T263_K	2	5.032	4.14	8.20	3.880	0.494			
T263_K	3	5.007	4.16	8.21	4.260	0.545			
T176_LED70	1	5.001	4.28	8.20	4.282	0.548	0.546	0.016	2.9
T176_LED70	2	5.013	4.14	8.20	4.388	0.560			
T176_LED70	3	5.001	4.45	8.20	4.134	0.529			
T176_LED140	1	5.008	4.35	8.20	4.288	0.548	0.543	0.005	1.0
T176_LED140	2	4.997	4.36	8.20	4.196	0.537			
T176_LED140	3	5.010	4.11	8.21	4.266	0.545			
T176_K	1	5.001	4.49	8.20	4.236	0.542	0.549	0.008	1.4
T176_K	2	4.995	4.51	8.20	4.274	0.548			
T176_K	3	4.997	4.47	8.20	4.350	0.557			

Treatment	Replicate	Mass of tomato (g)	pH initial	pH final	V NaOH (mL)	% TTA	Average % TTA	SD	CV %
Harvest 2									
T300_LED70	1	4.996	4.47	8.21	3.958	0.507	0.513	0.008	1.6
T300_LED70	2	5.002	4.38	8.21	3.986	0.510			
T300_LED70	3	5.009	4.32	8.20	4.090	0.523			
T300_K	1	5.003	4.14	8.21	3.726	0.477	0.477	0.013	2.7
T300_K	2	4.995	4.11	8.21	3.830	0.491			
T300_K	3	4.993	4.21	8.20	3.624	0.465			
T263_LED70	1	4.990	4.13	8.21	4.586	0.588	0.578	0.008	1.5
T263_LED70	2	4.996	3.87	8.21	4.482	0.574			
T263_LED70	3	4.937	3.68	8.21	4.420	0.573			
T263_K	1	5.004	4.42	8.20	4.044	0.517	0.521	0.013	2.5
T263_K	2	4.997	4.57	8.20	3.986	0.511			
T263_K	3	5.020	4.11	8.20	4.204	0.536			
T176_LED70	1	5.001	4.78	8.20	4.252	0.544	0.522	0.021	4.0
T176_LED70	2	4.997	4.84	8.20	3.930	0.503			
T176_LED70	3	5.008	4.54	8.20	4.054	0.518			
T176_LED140	1	5.026	4.21	8.20	3.908	0.498	0.501	0.003	0.5
T176_LED140	2	5.001	4.67	8.20	3.928	0.503			
T176_LED140	3	5.056	4.30	8.20	3.960	0.501			
T176_K	1	5.018	4.37	8.20	4.054	0.517	0.541	0.039	7.1
T176_K	2	4.952	4.41	8.20	4.534	0.586			
T176_K	3	4.932	4.46	8.20	4.016	0.521			
Harvest 3									
T300_LED70	1	4.983	4.65	8.20	4.050	0.520	0.535	0.017	3.1
T300_LED70	2	5.055	4.68	8.21	4.368	0.553			
T300_LED70	3	5.026	4.72	8.20	4.184	0.533			
T300_K	1	5.021	4.68	8.20	4.282	0.546	0.522	0.034	6.5
T300_K	2	4.961	4.52	8.20	4.166	0.537			
T300_K	3	4.972	4.92	8.21	3.754	0.483			
T263_LED70	1	4.993	4.32	8.21	3.988	0.511	0.508	0.004	0.8
T263_LED70	2	5.000	4.29	8.20	3.980	0.509			
T263_LED70	3	4.992	4.44	8.21	3.924	0.503			
T263_K	1	4.980	4.34	8.20	3.990	0.513	0.493	0.017	3.5
T263_K	2	4.991	3.98	8.21	3.780	0.485			
T263_K	3	5.029	4.15	8.20	3.778	0.481			
T176_LED70	1	5.015	4.44	8.20	3.890	0.496	0.497	0.008	1.6
T176_LED70	2	4.982	4.26	8.20	3.936	0.506			
T176_LED70	3	5.000	4.55	8.20	3.824	0.490			
T176_LED140	1	4.996	4.43	8.20	3.932	0.504	0.499	0.009	1.9
T176_LED140	2	5.010	4.27	8.21	3.822	0.488			
T176_LED140	3	4.992	4.52	8.21	3.942	0.505			
T176_K	1	4.938	4.40	8.20	3.818	0.495	0.505	0.028	5.6
T176_K	2	5.014	4.34	8.20	3.788	0.484			
T176_K	3	5.032	4.47	8.20	4.222	0.537			

Table B2 *The measurements of firmness and soluble solid content (SSC) in tomatoes grown under different light treatments at Nibio, Særheim in autumn 2018 / winter 2019*

Treatment	Replicate	Firmness	Average Firmness	SD	CV %	SSC (°Brix)	Average SSC (°Brix)	SD	CV %
Harvest 1									
T300_LED70	1	82	81.0	0.01	1.4	5.3	5.2	0.12	2.2
T300_LED70	2	80				5.1			
T300_LED70	3	80				5.3			
T300_K	1	82	81.0	0.01	1.4	5.1	5.1	0.00	0.0
T300_K	2	82				5.1			
T300_K	3	80				5.1			
T263_LED70	1	84	84.7	0.58	0.7	5.1	5.1	0.06	1.1
T263_LED70	2	85				5.2			
T263_LED70	3	85				5.1			
T263_K	1	83	81.7	1.15	1.4	5.1	5.1	0.06	1.1
T263_K	2	81				5.1			
T263_K	3	81				5.2			
T176_LED70	1	88	87.3	1.15	1.3	5.1	5.0	0.15	3.1
T176_LED70	2	88				4.8			
T176_LED70	3	86				5.0			
T176_LED140	1	87	86.7	0.58	0.7	4.9	4.8	0.17	3.6
T176_LED140	2	87				4.6			
T176_LED140	3	86				4.9			
T176_K	1	90	89.0	1.00	1.1	5.0	4.9	0.06	1.2
T176_K	2	88				4.9			
T176_K	3	89				4.9			
Harvest 2									
T300_LED70	1	83	83.0	0.00	0.0	5.3	5.3	0.00	0.0
T300_LED70	2	83				5.3			
T300_LED70	3	83				5.3			
T300_K	1	81	81.0	0.02	1.9	5.1	5.1	0.06	1.1
T300_K	2	83				5.1			
T300_K	3	80				5.0			
T263_LED70	1	87	86.7	0.58	0.7	5.2	5.2	0.10	1.9
T263_LED70	2	87				5.3			
T263_LED70	3	86				5.1			
T263_K	1	88	86.7	1.53	1.8	5.1	5.2	0.06	1.1
T263_K	2	85				5.2			
T263_K	3	87				5.2			
T176_LED70	1	90	89.3	0.58	0.6	4.8	4.8	0.10	2.1
T176_LED70	2	89				4.7			
T176_LED70	3	89				4.9			
T176_LED140	1	90	90.0	0.00	0.0	4.9	5.0	0.12	2.3
T176_LED140	2	90				4.9			
T176_LED140	3	90				5.1			
T176_K	1	91	90.7	1.53	1.7	4.6	4.7	0.12	2.5
T176_K	2	89				4.8			
T176_K	3	92				4.6			

Harvest 3									
T300_LED70	1	86	83.0	0.03	3.2	5.4	5.3	0.12	2.2
T300_LED70	2	82				5.4			
T300_LED70	3	81				5.2			
T300_K	1	84	84.0	0.01	0.7	4.9	5.1	0.17	3.4
T300_K	2	84				5.2			
T300_K	3	83				5.2			
T263_LED70	1	83	84.0	1.73	2.1	4.9	4.9	0.06	1.2
T263_LED70	2	83				4.9			
T263_LED70	3	86				4.8			
T263_K	1	85	85.0	1.00	1.2	4.9	5.1	0.20	3.9
T263_K	2	86				5.1			
T263_K	3	84				5.3			
T176_LED70	1	89	89.0	0.00	0.0	4.8	4.8	0.06	1.2
T176_LED70	2	89				4.7			
T176_LED70	3	89				4.8			
T176_LED140	1	86	87.7	2.08	2.4	5.0	4.9	0.10	2.0
T176_LED140	2	90				4.8			
T176_LED140	3	87				4.9			
T176_K	1	89	88.3	1.15	1.3	4.4	4.4	0.00	0.0
T176_K	2	89				4.4			
T176_K	3	87				4.4			

Table B3 *Dry weight (DW) in tomatoes grown under different light treatments at Nibio, Særheim in autumn2018 / winter 2019*

Treatment	Replicate	DW (%)	Average DW (%)	SD	CV (%)
Harvest 2					
T300_LED70	1	6.36	6.50	0.25	3.8
T300_LED70	2	6.36			
T300_LED70	3	6.79			
T300_K	1	6.43	6.42	0.03	0.5
T300_K	2	6.44			
T300_K	3	6.38			
T263_LED70	1	6.79	6.82	0.02	0.4
T263_LED70	2	6.84			
T263_LED70	3	6.83			
T263_K	1	6.68	6.73	0.12	1.8
T263_K	2	6.65			
T263_K	3	6.87			
T176_LED70	1	3.89	6.57	2.65	40.3
T176_LED70	2	9.19			
T176_LED70	3	6.64			
T176_LED140	1	6.36	6.57	0.29	4.4
T176_LED140	2	6.46			
T176_LED140	3	6.90			
T176_K	1	5.86	5.93	0.11	1.8
T176_K	2	6.06			
T176_K	3	5.89			

ANOVA analysis – experiment 2

Experiment 2, Total titratable acidity, Harvest 1

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, TTA, H1

Normality Test (Shapiro-Wilk): Passed (P = 0.788)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.359)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	0.549	0.00759	0.00438
T176_LED70	6	3	0.546	0.0157	0.00908
T176_LED140	6	3	0.543	0.00546	0.00315
T263_K	6	3	0.516	0.0261	0.0151
T263_LED70	6	3	0.552	0.0129	0.00742
T300_K	6	3	0.546	0.00323	0.00186
T300_LED70	6	3	0.558	0.0188	0.0108

Source of Variation	DF	SS	MS	F	P
Between Groups	6	0.00329	0.000548	2.486	0.075
Residual	14	0.00309	0.000221		
Total	20	0.00638			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.075).

Power of performed test with alpha = 0.050: 0.399

The power of the performed test (0.399) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Experiment 2, Total titratable acidity, Harvest 2

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, TTA, H2

Normality Test (Shapiro-Wilk): Passed (P = 0.115)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.636)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	0.541	0.0387	0.0223
T176_LED70	6	3	0.522	0.0207	0.0119
T176_LED140	6	3	0.501	0.00264	0.00152
T263_K	6	3	0.521	0.0132	0.00763
T263_LED70	6	3	0.578	0.00845	0.00488
T300_K	6	3	0.477	0.0131	0.00757
T300_LED70	6	3	0.513	0.00828	0.00478

Source of Variation	DF	SS	MS	F	P
Between Groups	6	0.0183	0.00305	8.844	<0.001
Residual	14	0.00483	0.000345		
Total	20	0.0231			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.994

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T263_LED70 vs. T300_K	0.101	6.671	<0.001	Yes
T263_LED70 vs. T176_LED140	0.0779	5.138	0.003	Yes
T263_LED70 vs. T300_LED70	0.0653	4.303	0.014	Yes
T176_K vs. T300_K	0.0641	4.228	0.015	Yes
T263_LED70 vs. T263_K	0.0572	3.773	0.034	Yes
T263_LED70 vs. T176_LED70	0.0566	3.733	0.035	Yes
T176_LED70 vs. T300_K	0.0446	2.938	0.150	No
T263_K vs. T300_K	0.0440	2.898	0.152	No
T176_K vs. T176_LED140	0.0409	2.694	0.205	No
T263_LED70 vs. T176_K	0.0371	2.444	0.292	No
T300_LED70 vs. T300_K	0.0359	2.369	0.307	No
T176_K vs. T300_LED70	0.0282	1.859	0.585	No
T176_LED140 vs. T300_K	0.0233	1.534	0.762	No
T176_LED70 vs. T176_LED140	0.0213	1.404	0.800	No
T263_K vs. T176_LED140	0.0207	1.365	0.779	No
T176_K vs. T263_K	0.0202	1.329	0.747	No
T176_K vs. T176_LED70	0.0196	1.290	0.708	No
T300_LED70 vs. T176_LED140	0.0127	0.835	0.885	No
T176_LED70 vs. T300_LED70	0.00863	0.569	0.925	No
T263_K vs. T300_LED70	0.00803	0.530	0.844	No
T176_LED70 vs. T263_K	0.000600	0.0396	0.969	No

Experiment 2, Total titratable acidity, Harvest 3

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, TTA, H3

Normality Test (Shapiro-Wilk): Passed (P = 0.972)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.642)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	0.505	0.0282	0.0163
T176_LED70	6	3	0.497	0.00808	0.00466
T176_LED140	6	3	0.499	0.00948	0.00547
T263_K	6	3	0.493	0.0175	0.0101
T263_LED70	6	3	0.508	0.00425	0.00246
T300_K	6	3	0.522	0.0340	0.0196
T300_LED70	6	3	0.535	0.0165	0.00955

Source of Variation	DF	SS	MS	F	P
Between Groups	6	0.00415	0.000691	1.791	0.173
Residual	14	0.00540	0.000386		
Total	20	0.00955			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.173).

Power of performed test with alpha = 0.050: 0.218

The power of the performed test (0.218) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Experiment 2, Soluble solid content, Harvest 1

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, SSC, H1

Normality Test (Shapiro-Wilk): Passed (P = 0.074)

Equal Variance Test (Brown-Forsythe): Passed (P = 1.000)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	4.933	0.0577	0.0333
T176_LED70	6	3	4.967	0.153	0.0882
T176_LED140	6	3	4.800	0.173	0.100
T263_K	6	3	5.133	0.0577	0.0333
T263_LED70	6	3	5.133	0.0577	0.0333
T300_K	6	3	5.100	0.000	0.000
T300_LED70	6	3	5.233	0.115	0.0667

Source of Variation	DF	SS	MS	F	P
Between Groups	6	0.398	0.0663	6.058	0.003
Residual	14	0.153	0.0110		
Total	20	0.551			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003).

Power of performed test with alpha = 0.050: 0.936

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T300_LED70 vs. T176_LED140	0.433	5.071	0.004	Yes
T263_LED70 vs. T176_LED140	0.333	3.901	0.031	Yes
T263_K vs. T176_LED140	0.333	3.901	0.030	Yes
T300_LED70 vs. T176_K	0.300	3.511	0.060	No
T300_K vs. T176_LED140	0.300	3.511	0.057	No
T300_LED70 vs. T176_LED70	0.267	3.121	0.114	No
T263_K vs. T176_K	0.200	2.341	0.410	No
T263_LED70 vs. T176_K	0.200	2.341	0.389	No
T263_K vs. T176_LED70	0.167	1.950	0.618	No
T263_LED70 vs. T176_LED70	0.167	1.950	0.589	No
T176_LED70 vs. T176_LED140	0.167	1.950	0.557	No
T300_K vs. T176_K	0.167	1.950	0.523	No
T300_K vs. T176_LED70	0.133	1.560	0.745	No
T176_K vs. T176_LED140	0.133	1.560	0.704	No
T300_LED70 vs. T300_K	0.133	1.560	0.655	No
T300_LED70 vs. T263_K	0.100	1.170	0.838	No
T300_LED70 vs. T263_LED70	0.100	1.170	0.780	No
T263_LED70 vs. T300_K	0.0333	0.390	0.992	No
T263_K vs. T300_K	0.0333	0.390	0.974	No
T176_LED70 vs. T176_K	0.0333	0.390	0.911	No
T263_K vs. T263_LED70	0.000	0.000	1.000	No

Experiment 2, Soluble solid content, Harvest 2

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, SSC, H2

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	4.667	0.115	0.0667
T176_LED70	6	3	4.800	0.100	0.0577
T176_LED140	6	3	4.967	0.115	0.0667
T263_K	6	3	5.167	0.0577	0.0333
T263_LED70	6	3	5.200	0.100	0.0577
T300_K	6	3	5.067	0.0577	0.0333
T300_LED70	6	3	5.300	0.000	0.000

Source of Variation	DF	SS	MS	F	P
Between Groups	6	0.931	0.155	20.375	<0.001
Residual	14	0.107	0.00762		
Total	20	1.038			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T300_LED70 vs. T176_K	0.633	8.886	<0.001	Yes
T263_LED70 vs. T176_K	0.533	7.483	<0.001	Yes
T263_K vs. T176_K	0.500	7.016	<0.001	Yes
T300_LED70 vs. T176_LED70	0.500	7.016	<0.001	Yes
T263_LED70 vs. T176_LED70	0.400	5.612	0.001	Yes
T300_K vs. T176_K	0.400	5.612	0.001	Yes
T263_K vs. T176_LED70	0.367	5.145	0.002	Yes
T300_LED70 vs. T176_LED140	0.333	4.677	0.005	Yes
T176_LED140 vs. T176_K	0.300	4.209	0.011	Yes
T300_K vs. T176_LED70	0.267	3.742	0.026	Yes
T300_LED70 vs. T300_K	0.233	3.274	0.059	No
T263_LED70 vs. T176_LED140	0.233	3.274	0.054	No
T263_K vs. T176_LED140	0.200	2.806	0.119	No
T176_LED140 vs. T176_LED70	0.167	2.339	0.246	No
T263_LED70 vs. T300_K	0.133	1.871	0.452	No
T176_LED70 vs. T176_K	0.133	1.871	0.403	No
T300_LED70 vs. T263_K	0.133	1.871	0.350	No
T263_K vs. T300_K	0.100	1.403	0.553	No
T300_K vs. T176_LED140	0.1000	1.403	0.453	No
T300_LED70 vs. T263_LED70	0.1000	1.403	0.331	No
T263_LED70 vs. T263_K	0.0333	0.468	0.647	No

Experiment 2, Soluble solid content, Harvest 3

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, SSC, H3

Normality Test (Shapiro-Wilk): Passed (P = 0.267)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	4.400	0.000	0.000
T176_LED70	6	3	4.767	0.0577	0.0333
T176_LED140	6	3	4.900	0.100	0.0577
T263_K	6	3	5.100	0.200	0.115
T263_LED70	6	3	4.867	0.0577	0.0333
T300_K	6	3	5.100	0.173	0.1000
T300_LED70	6	3	5.333	0.115	0.0667

Source of Variation	DF	SS	MS	F	P
Between Groups	6	1.598	0.266	18.644	<0.001
Residual	14	0.200	0.0143		
Total	20	1.798			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T300_LED70 vs. T176_K	0.933	9.564	<0.001	Yes
T300_K vs. T176_K	0.700	7.173	<0.001	Yes
T263_K vs. T176_K	0.700	7.173	<0.001	Yes
T300_LED70 vs. T176_LED70	0.567	5.807	<0.001	Yes
T176_LED140 vs. T176_K	0.500	5.123	0.003	Yes
T263_LED70 vs. T176_K	0.467	4.782	0.005	Yes
T300_LED70 vs. T263_LED70	0.467	4.782	0.004	Yes
T300_LED70 vs. T176_LED140	0.433	4.440	0.008	Yes
T176_LED70 vs. T176_K	0.367	3.757	0.027	Yes
T263_K vs. T176_LED70	0.333	3.416	0.049	Yes
T300_K vs. T176_LED70	0.333	3.416	0.045	Yes
T263_K vs. T263_LED70	0.233	2.391	0.273	No
T300_K vs. T263_LED70	0.233	2.391	0.250	No
T300_LED70 vs. T300_K	0.233	2.391	0.225	No
T300_LED70 vs. T263_K	0.233	2.391	0.200	No
T300_K vs. T176_LED140	0.200	2.049	0.309	No
T263_K vs. T176_LED140	0.200	2.049	0.265	No
T176_LED140 vs. T176_LED70	0.133	1.366	0.577	No
T263_LED70 vs. T176_LED70	0.100	1.025	0.690	No
T176_LED140 vs. T263_LED70	0.0333	0.342	0.931	No
T263_K vs. T300_K	0.000	0.000	1.000	No

Experiment 2, Firmness, Harvest 1

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, Firmness, H1

Normality Test (Shapiro-Wilk): Passed (P = 0.087)

Equal Variance Test (Brown-Forsythe): Passed (P = 1.000)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	89.000	1.000	0.577
T176_LED70	6	3	87.333	1.155	0.667
T176_LED140	6	3	86.667	0.577	0.333
T263_K	6	3	81.667	1.155	0.667
T263_LED70	6	3	84.667	0.577	0.333
T300_K	6	3	81.333	1.155	0.667
T300_LED70	6	3	80.667	1.155	0.667

Source of Variation	DF	SS	MS	F	P
Between Groups	6	197.238	32.873	32.873	<0.001
Residual	14	14.000	1.000		
Total	20	211.238			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T176_K vs. T300_LED70	8.333	10.206	<0.001	Yes
T176_K vs. T300_K	7.667	9.390	<0.001	Yes
T176_K vs. T263_K	7.333	8.981	<0.001	Yes
T176_LED70 vs. T300_LED70	6.667	8.165	<0.001	Yes
T176_LED140 vs. T300_LED70	6.000	7.348	<0.001	Yes
T176_LED70 vs. T300_K	6.000	7.348	<0.001	Yes
T176_LED70 vs. T263_K	5.667	6.940	<0.001	Yes
T176_LED140 vs. T300_K	5.333	6.532	<0.001	Yes
T176_LED140 vs. T263_K	5.000	6.124	<0.001	Yes
T176_K vs. T263_LED70	4.333	5.307	0.001	Yes
T263_LED70 vs. T300_LED70	4.000	4.899	0.003	Yes
T263_LED70 vs. T300_K	3.333	4.082	0.011	Yes
T263_LED70 vs. T263_K	3.000	3.674	0.022	Yes
T176_LED70 vs. T263_LED70	2.667	3.266	0.044	Yes
T176_K vs. T176_LED140	2.333	2.858	0.085	No
T176_LED140 vs. T263_LED70	2.000	2.449	0.157	No
T176_K vs. T176_LED70	1.667	2.041	0.268	No
T263_K vs. T300_LED70	1.000	1.225	0.668	No
T176_LED70 vs. T176_LED140	0.667	0.816	0.813	No
T300_K vs. T300_LED70	0.667	0.816	0.673	No
T263_K vs. T300_K	0.333	0.408	0.689	No

Experiment 2, Firmness, Harvest 2

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, Firmness, H2

Normality Test (Shapiro-Wilk): Passed (P = 0.159)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.716)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	90.667	1.528	0.882
T176_LED70	6	3	89.333	0.577	0.333
T176_LED140	6	3	90.000	0.000	0.000
T263_K	6	3	86.667	1.528	0.882
T263_LED70	6	3	86.667	0.577	0.333
T300_K	6	3	81.333	1.528	0.882
T300_LED70	6	3	83.000	0.000	0.000

Source of Variation	DF	SS	MS	F	P
Between Groups	6	227.905	37.984	34.681	<0.001
Residual	14	15.333	1.095		
Total	20	243.238			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T176_K vs. T300_K	9.333	10.923	<0.001	Yes
T176_LED140 vs. T300_K	8.667	10.142	<0.001	Yes
T176_LED70 vs. T300_K	8.000	9.362	<0.001	Yes
T176_K vs. T300_LED70	7.667	8.972	<0.001	Yes
T176_LED140 vs. T300_LED70	7.000	8.192	<0.001	Yes
T176_LED70 vs. T300_LED70	6.333	7.412	<0.001	Yes
T263_K vs. T300_K	5.333	6.242	<0.001	Yes
T263_LED70 vs. T300_K	5.333	6.242	<0.001	Yes
T176_K vs. T263_LED70	4.000	4.681	0.005	Yes
T176_K vs. T263_K	4.000	4.681	0.004	Yes
T263_K vs. T300_LED70	3.667	4.291	0.008	Yes
T263_LED70 vs. T300_LED70	3.667	4.291	0.007	Yes
T176_LED140 vs. T263_K	3.333	3.901	0.014	Yes
T176_LED140 vs. T263_LED70	3.333	3.901	0.013	Yes
T176_LED70 vs. T263_LED70	2.667	3.121	0.051	No
T176_LED70 vs. T263_K	2.667	3.121	0.044	Yes
T300_LED70 vs. T300_K	1.667	1.950	0.310	No
T176_K vs. T176_LED70	1.333	1.560	0.455	No
T176_K vs. T176_LED140	0.667	0.780	0.832	No
T176_LED140 vs. T176_LED70	0.667	0.780	0.696	No
T263_K vs. T263_LED70	0.000	0.000	1.000	No

Experiment 2, Firmness, Harvest 3

One Way Analysis of Variance – Report from SigmaPlot 14.0

Data source: Experiment 2, Firmness, H3

Normality Test (Shapiro-Wilk): Passed (P = 0.250)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.461)

Group Name	N	Missing	Mean	Std Dev	SEM
T176_K	6	3	88.333	1.155	0.667
T176_LED70	6	3	89.000	0.000	0.000
T176_LED140	6	3	87.667	2.082	1.202
T263_K	6	3	85.000	1.000	0.577
T263_LED70	6	3	84.000	1.732	1.000
T300_K	6	3	83.667	0.577	0.333
T300_LED70	6	3	83.000	2.646	1.528

Source of Variation	DF	SS	MS	F	P
Between Groups	6	109.238	18.206	7.497	<0.001
Residual	14	34.000	2.429		
Total	20	143.238			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.980

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
T176_LED70 vs. T300_LED70	6.000	4.715	0.007	Yes
T176_LED70 vs. T300_K	5.333	4.191	0.018	Yes
T176_K vs. T300_LED70	5.333	4.191	0.017	Yes
T176_LED70 vs. T263_LED70	5.000	3.930	0.027	Yes
T176_LED140 vs. T300_LED70	4.667	3.668	0.042	Yes
T176_K vs. T300_K	4.667	3.668	0.040	Yes
T176_K vs. T263_LED70	4.333	3.406	0.062	No
T176_LED140 vs. T300_K	4.000	3.144	0.096	No
T176_LED70 vs. T263_K	4.000	3.144	0.089	No
T176_LED140 vs. T263_LED70	3.667	2.882	0.136	No
T176_K vs. T263_K	3.333	2.620	0.201	No
T176_LED140 vs. T263_K	2.667	2.096	0.431	No
T263_K vs. T300_LED70	2.000	1.572	0.738	No
T263_K vs. T300_K	1.333	1.048	0.950	No
T176_LED70 vs. T176_LED140	1.333	1.048	0.927	No
T263_K vs. T263_LED70	1.000	0.786	0.971	No
T263_LED70 vs. T300_LED70	1.000	0.786	0.947	No
T300_K vs. T300_LED70	0.667	0.524	0.977	No
T176_LED70 vs. T176_K	0.667	0.524	0.940	No
T176_K vs. T176_LED140	0.667	0.524	0.847	No
T263_LED70 vs. T300_K	0.333	0.262	0.797	No

Appendix C

Calibration curves for quantitative determination of citric and malic acid

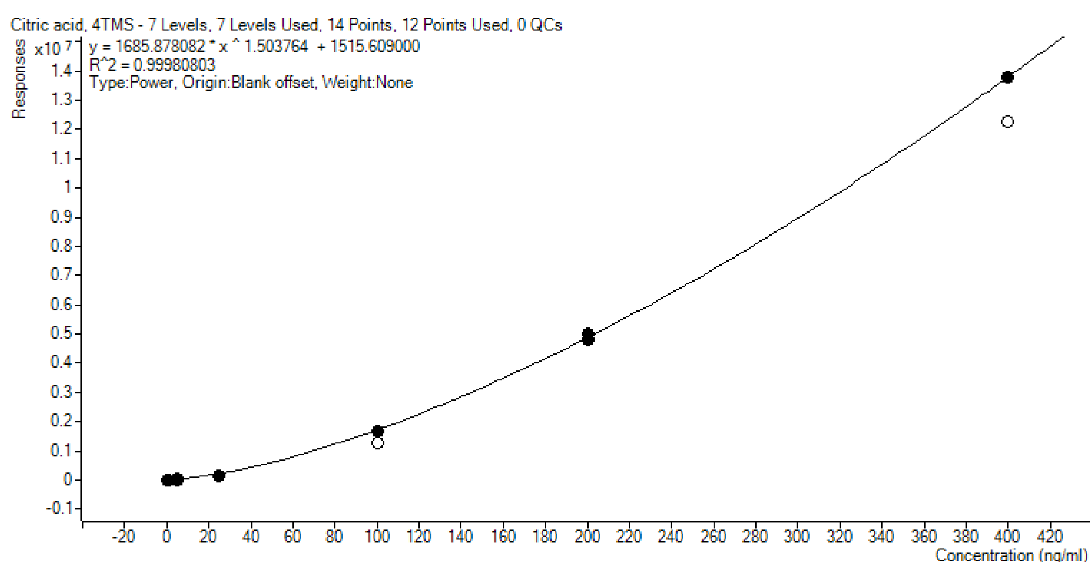

Figure C1 *Calibration curve for citric acid*

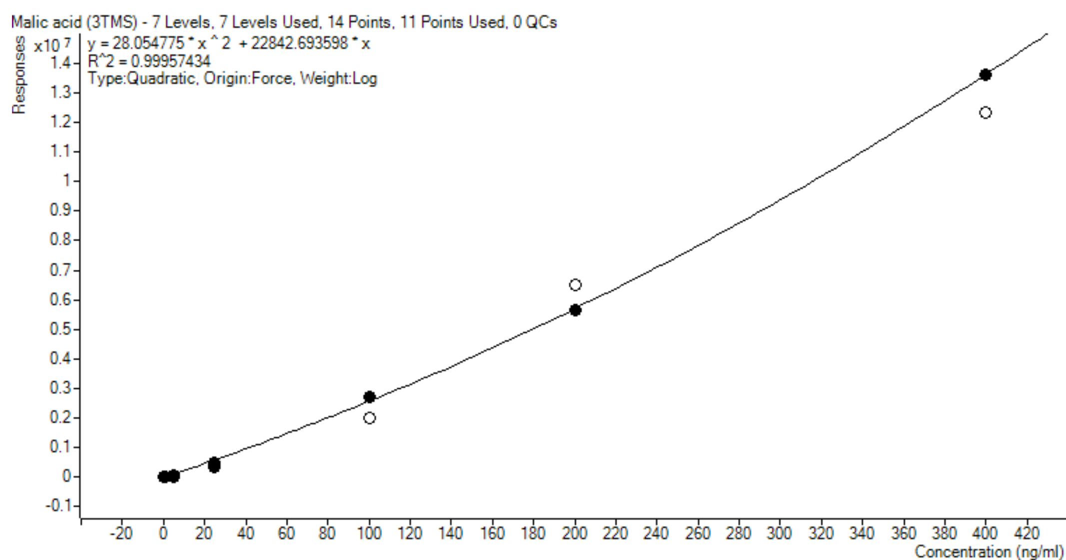

Figure C2 *Calibration curve for malic acid*

Scree plot from principal component analysis

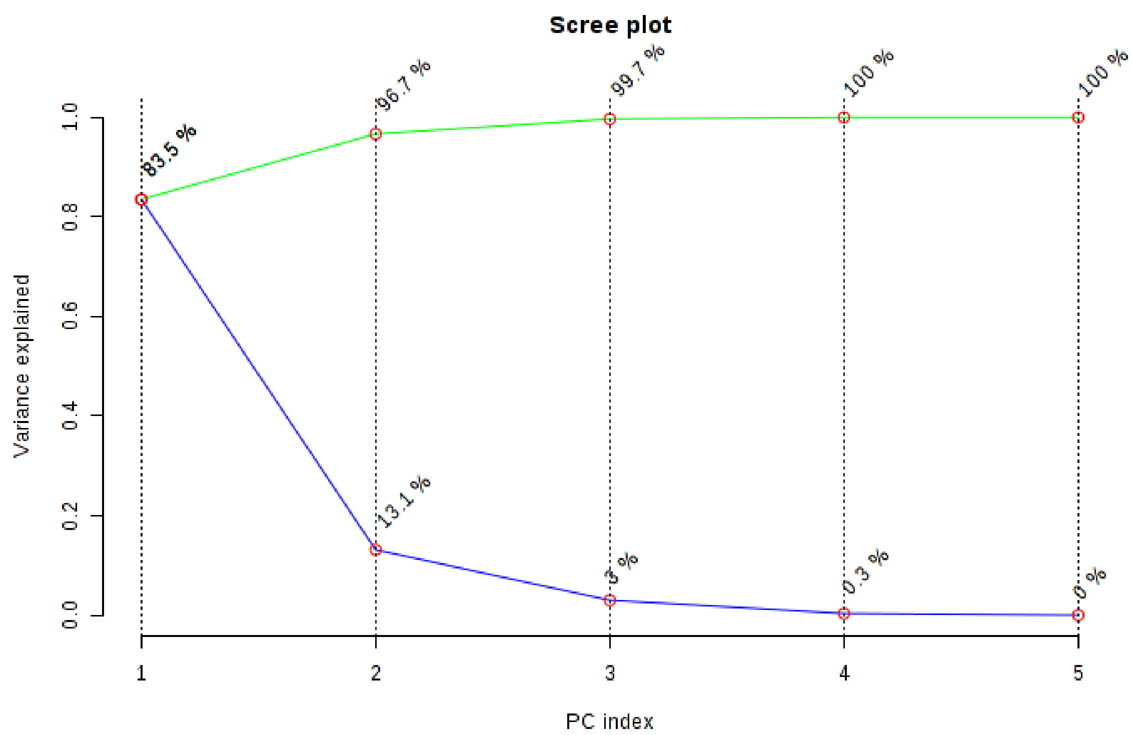

Figure C3 *PCA scree plot showing percentage of variance that is captured from the data by each principal component. PCA was performed on the samples from tomatoes grown under different nutrient conditions (experiment 1)*