POTENTIAL OF PRE- AND POSTHARVEST ILLUMINATION OF CHERRY TOMATO, A CLIMACTERIC FRUIT, TO REDUCE THE RIPENING PERIOD AND ENHANCE YIELD AND QUALITY WHILE MAINTAINING SHELF LIFE

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DECLARATION

The research in this thesis was as a result of the experiments carried out in the School of Agriculture, Earth and Environmental Science, University of KwaZulu-Natal, Pietermaritzburg from February 2017 to October 2017.

I hereby declare that the research reported in this project write up is my own, unless otherwise indicated and referenced accordingly.

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DEDICATION

This dissertation is dedicated to ***MTARABHI***, my first born

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LIST OF ACRONYMS

AsA, Ascorbic acid

BL, Blue Light

CA, Controlled Atmosphere

Chl a, Chlorophyll a

Chl b, Chlorophyll b

FR, Far Red

GF, Green Fluorescence

GL, Green Light

HID, High-Intensity Discharge

HPS- High Pressure Sodium

HSPs- Heat Shock Proteins

LAI, Leaf Area Index

LEDs, Light Emitting Diodes

MAP, Modified Atmosphere packaging

MH, Metal halide

Pfr, Phytochrome far-red

PAR, Photosynthetically active radiation

PPF, Photosynthetic Photon Flux

RB, Red and Blue

RBW, Red, Blue and White

RGB, Red, Green and Blue

RL, Red Light

TSS, Total Soluble Solids

UV-B, Ultraviolet B

UV-C, Ultraviolet C

ABSTRACT

Tomato (*Solanum lycopersicum*) is the most-consumed horticultural commodity worldwide because it is diverse in use, attractive and contributes significantly to the health and nutrition of humans. There are many different types of tomato cultivars, such as the classic round, plum and baby plum, cherry, beefsteak, vine or truss and cocktail tomatoes. Baby tomatoes, also termed 'cherry tomatoes', have become particularly popular as fruit vegetables, due to their taste, particularly sweetness, high nutritional value and health benefits, as well as their attractive colour, particularly in the presentation of food. Many horticultural commodities are nowadays cultivated under supplemental lighting, such as ultraviolet C (UV-C), light emitting diodes (LEDs), and high-pressure sodium (HPS) so as to improve yield and reduce ripening period since the demand of tomato, particularly cherry tomato is increasing significantly which forces tomato growers to make use of controlled environment to meet the increasing demand. The use of LEDs in protected cultivation is gaining popularity as it can improve yields and enhance certain phytochemicals. Light-emitting diodes (LEDs) represent a relatively new technology for the greenhouse industry, as they emit light of narrow bandwidths.

These lights are affordable and they do not contain unnecessary, low quality wavelengths. Therefore, LEDs can be employed to promote growth of fruit and vegetables in agriculture, particularly in horticulture, as they aid in plant development. Further, LEDs are easily controllable light sources and their use can improve the nutritional content of certain commodities, while improving or maintaining yield and giving high quality produce. Light affect the presence of phytonutrients in tomato fruit, such as carotenoids, vitamin C and phenolics. The general aim of this study was to determine if certain treatments are able to fast-forward colour change, while maintaining the fruit quality of cherry tomato.

Two experiments were conducted, one in the glasshouse and another one in the post-harvest laboratory at the University of KwaZulu-Natal in 2017. The first experiment was designed to evaluate the effect of pre-harvest red and blue light treatment on colour, ripening, chlorophyll and carotenoid concentration as well as overall quality of the cherry tomato cultivars ('Cherry Little Wonders' and 'Goldilocks'). When fruit were mature green, the a^* values of the twelve trusses of the same age, six from each cultivar, were selected to receive light treatment. Six trusses, three of each cultivar, were illuminated with FLC-10W-R Red LED light (RL) and

another six trusses, three of each cultivar, were illuminated with FLC-10W-BL blue LED light (BL). It was ensured that the distance from each light source to the truss was the same and it was also ensured that the light was equally distributed to every truss. Certain fruit were marked in each truss for analysis of quality parameters or measurements such as colour, size, firmness, TSS, chlorophyll and carotenoids.

In this study pre-harvest red and blue light significantly affected the measured quality attributes of two cultivars ('Cherry Little Wonders' and 'Goldilocks'), a red and yellow cherry tomatoes respectively. Light treatments did not have a significant effect on fruit size ($P > 0.05$) The size of all light-treated fruit was bigger than that of untreated fruit from day 15 to day 25, however there was no statistical significant difference between treated and non-treated fruits ($P > 0.05$). Yellow cultivar had a lower a* value and higher value of b*(green to yellow) from day 10 to day 25. A steady decrease in colour b* was observed in red cv while a sharp increase was observed in yellow cv, but fruits that were illuminated with red light had a higher b* value on both cultivars. Following treatment, L* (lightness) steadily decreased in treated and untreated tomato fruit for the first 10 days. Thereafter, a rapid decrease in L* was observed. A sharp decrease in chlorophyll concentration and a corresponding increase in carotenoid synthesis during the fruit ripening process was observed

Chlorophyll a, b and carotenoid concentrations in tomato differed significantly ($P < 0.01$) between treatments, with the control maintaining the highest Chl a and Chl b values until day 25. There was a statistical significant difference between untreated and treated fruit in terms of changes in Chl a and b ($P < 0.05$). The red cv treated with BL and the yellow cv treated with RL showed a rapid decrease in Chl a. The accumulation of lycopene commenced in treated tomatoes 10 days after treatment, but for the first 10 days there was no statistical difference between the treated and non-treated fruit ($P < 0.05$). The lycopene concentration of yellow tomatoes was lower that of red tomatoes. The firmness of treated and non-treated fruit was similar the same in all fruit for the first five days postharvest, except in the yellow cv treated with BL. This treatment lost firmness most rapidly. Light also prevented the occurrence of diseases and disorder.

The second study was conducted to investigate the effects of post-harvest red and blue LED light treatments on two cultivars of cherry tomatoes, red ('Cherry Little Wonders') and yellow ('Goldilocks') which received light at different stages of development, while on the plant as well as postharvest. The response of tomato cultivars that received post-harvest light treatment did not differ significantly with the cultivar that was treated and allowed to ripen on the tree. Light treatments were able to enhance colour development more on cherry tomato fruits treated at mature green compared to those treated at turning stage.

The effect of light on chlorophyll a and b on fruits varied according to the cultivars. Fruit that were treated at turning stage had lower chlorophylls initially and then a steady rate of change was observed while a sharp/rapid degradation of chlorophylls was observed in fruits treated at mature green. Light effects on degradation of chlorophylls had no significant difference within the stage at which plants received the treatment. Lycopene was the major pigment in red cv of cherry tomatoes. It was influenced equally by red and blue lights, with fruit treated at mature green had more lycopene that those treated at turning stage. There was a significant difference between treatments and the control in terms of lycopene and β-carotene content which were higher in fruits treated at mature green.

There was no significant difference ($P < 0.05$) in change in mass of fruit that received red and blue lights and non-treated fruits meaning that light did not have a negative effect on tomato fruits treated at mature green stage and at turning stage. Light treatments were able to prevent the occurrence of diseases on all the treatments.

Keywords: **carotenoids, cherry tomato, glasshouse, health benefits, light emitting diodes (LEDs)**

CHAPTER ONE

GENERAL INTRODUCTION

The need for production of vegetables with high aesthetic value and high nutritional quality has increased significantly in recent years, particularly due to the health benefits related to the consumption of these commodities (Ribeiro et al., 2012). Tomato (*Solanum lycopersicum*) is the most-consumed horticultural commodity worldwide because it is diverse in use, attractive and contributes significantly to the health and nutrition of humans (Soto-Zamora et al., 2005). There are many different types of tomato cultivars, such as the classic round, plum and baby plum, cherry, beefsteak, vine or truss and cocktail tomatoes. About 6 000 hectares are planted with tomatoes in South Africa, resulting in an annual production of 152 million tons in 2012, whereas the production was only 89.9 million (FAOSTAT, 2013), making tomatoes the most important non-starch vegetable in the country. Tomatoes are, however, also grown by resource-poor farmers, home gardeners and subsistence farmers. The latter grow tomatoes for their own consumption and for small-scale sale. Tomatoes contribute about 24% to the total vegetable production in South Africa (DAFF, 2015), while the crop contributes approximately 20% to the gross value of vegetable production globally (excluding potatoes) (FAOSTAT, 2015).

Tomato is a climacteric and very perishable fruit that is highly susceptible to microbial infection because of the rapid ripening at ambient conditions (Maharaj et al., 1999). Tomatoes can be consumed fresh, in salads, or as an ingredient in foods, like hamburgers and pizzas, as fresh juice or as a canned product. Tomatoes are also recognized as a good source of ascorbic acid, and carotenoids, particularly β-carotene and lycopene (Mangels et al., 1993). Together with the attractive colour of the fruit, these features have led to an increased consumption of tomatoes over the past five years (FAOSTAT, 2015). Baby tomatoes, also termed ‘cherry tomatoes’, have become particularly popular as fruit vegetables, due to their taste, particularly sweetness, high nutritional value and health benefits, as well as their attractive colour, particularly in the presentation of food (Giovannucci, 1999; Rosales et al., 2006). Carotenoids in tomato play, therefore, a significant role in the prevention of various diseases, such as cancer, cataracts, and heart disease (Agarwal and Rao, 2000).

In order to achieve optimal yields in cherry tomato, a very high light fluence-rate is required in large quantities. The amount of light and the quality of light influences the rate of photosynthesis and growth of the tomato plant (Kinet, 1977). The use of artificial lights, such as fluorescent lamps and light emitting diodes (LEDs), has become popular lately, with many garden crops grown commercially with such lights. LEDs have become especially popular for the cultivation of vegetable crops (Hoenecke et al., 1992; Tennessen et al., 1994; Goins et al., 1997; Schuerger et al., 1997; Amaki and Hirai, 2008).

Light is the primary source of energy and allows the manipulation of plant growth and development (Massa et al., 2008). The two parameters of light, intensity and quality, influenced growth, morphogenesis and other physiological responses of plants (Fukuda et al., 2008; Li and Kubota, 2009). To determine the effect of light on plant growth, LEDs are employed, as they have several unique properties, such as producing high light intensity, but excluding heat that is commonly given off by incandescent lights. Further, LEDs have a narrow bandwidth, allowing the control of wavelength composition very specifically (Bourget, 2008). Quality, duration and intensity of light affect the presence of phytonutrients in tomato fruit, such as carotenoids, vitamin C and phenolics. According to previous studies, an increase in light intensity results in an increase in tomato fruit antioxidants, such as lycopene, β-carotene, vitamin C and phenolics (Ju et al., 1999; Lee and Kader, 2000; Merzlyak et al., 2002). While the biosynthesis of anthocyanins in anthocyanin-accumulating fruit is dependent on light (Lancaster, 1992), carotenoids do not necessarily need induction by light (Lintig et al., 1977). The quality of light is also a crucial determinant of nutritional quality of tomato fruit (Tomás-Barberán and Espin, 2001).

Skin colour and texture are some of the most important tomato fruit quality attributes from the consumer's perspective (Liu et al., 2011). The first characteristic that determines the degree of consumer acceptance is colour, while the final quality parameter, which consumers judge tomatoes on, is firmness; this parameter ultimately makes the consumer decide to buy fresh tomatoes (Pinheiro et al., 2013). The most crucial factors in the tomato purchasing decision are flavour, colour, taste and health benefits (León-Sánchez et al., 2009); in the last decades, however, commercial tomatoes have been criticized by consumers for lacking desirable taste and flavour (Krumbein et al., 2004).

In maintaining fruit quality and extending shelf-life, application of synthetic oils and waxes minimizes diffusion of gases and lowers diffusion of water out of the fruit. This, hence, creates a modified internal atmosphere inside the fruit, resulting, if maintained too long, in fermentation, as detectable by the release of offensive odours (off-flavours). Another technique that is commonly used to delay fruit ripening, as well as associated biochemical and physiological changes, is altering O_2 and CO_2 levels around the product using modified atmosphere packaging (MAP). Such MAP storage, as well as controlled atmosphere (CA) storage, where gas of a certain composition is released into a storage container, is used to increase the shelf life of fruit and vegetables. The use of MAP alters the gaseous environment around the commodity, as fruits are respiring, thereby reducing the O_2 in the packages, while simultaneously increasing the CO_2 due to respiration (passive MAP) or by the addition and removal of gases from food packages (active MAP) to manipulate O_2 and CO_2 levels. Reduced O_2 and/or elevated CO_2 levels reduce respiration, delay ripening, decrease ethylene production, retard textural softening, and slow down compositional changes associated with ripening, thereby resulting in an extension of shelf life (Daş et al., 2006).

The general aim of this study was to determine if certain treatments are able to fast-forward colour change, while maintaining the fruit quality of cherry tomato.

The specific objectives relevant to this study included the following:

- To investigate the role of LED light exposure to induce colour change in cherry tomatoes
- To determine the effectiveness of different LEDs in reducing the ripening period and enhancing yield, while maintaining or altering cherry tomato quality attributes
- To compare the effectiveness of LEDs on tomato treated at different stages of development

This study therefore, was conducted to enhance colour development and maintain fruit quality of tomatoes by exposing fruit, pre- and postharvest, to light, and packing fruit into modified perforated plastic packaging.

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CHAPTER TWO

THE EFFECT OF LIGHT EMITTING DIODES (LEDs) AND OTHER LIGHT SOURCES ON HORTICULTURAL COMMODITIES WITH SPECIAL REFERENCE TO TOMATO - Literature review

2.1 INTRODUCTION

Supplemental lighting is a tool used in the production of plants in controlled environments as it facilitates the growth of the plants and allows year-round production of tomatoes and other horticultural commodities of high quality (Kozai, 2007). Many horticultural commodities are nowadays cultivated under supplemental lighting, such as ultraviolet C (UV-C), light emitting diodes (LEDs), and high-pressure sodium (HPS). Tomato (*Solanum lycopersicum*) is one of the most important climacteric fruit, as it is consumed worldwide due to its attractiveness and numerous health benefits. China produces more tomato than any other country throughout the year (FAOSTAT, 2016). The demand of tomato particularly cherry tomato is increasing significantly which forces tomato growers to make use of controlled environments to meet the increasing demand. The use of LEDs in protected cultivation is gaining popularity as it can improve yields and enhance certain phytochemicals (Hoenecke et al., 1992; Tennessen et al., 1994; Goins et al., 1997; Schuerger et al., 1997; Kim et al., 2004a, 2004b; Amaki and Hirai, 2008). The effect of light has been studied on lettuce (Yorio et al., 2001; Kim et al., 2004d; Brazaitytė et al., 2006), radish (Yorio et al., 2001), spinach (Yorio et al., 2001), pepper (Schuerger et al., 1997), tomato (Kaneko-Ohashi et al., 2004d; Menard et al., 2005) and strawberry (Yanagi et al., 2006). This includes light-emitting diode systems that are most often based on blue, red and far-red LEDs (Schuerger et al., 1997; Lian et al., 2002; Jao and Fang, 2004; Matsuda et al., 2004). Alternatively, hybrid illumination, such as fluorescent light supplemented by red or blue LEDs (Schuerger et al., 1997; Yorio et al., 2001; Topchiy et al., 2005; Menard et al., 2005) can be used. LEDs are currently the most vital technology to affect fruit growth due to their unique capabilities and lend themselves well to shelf lighting, particularly due to their low radiant heat output.

The use of various light sources, such as LEDs, UV-C, HPS, fluorescent and incandescent lights, can be employed to enhance plant and fruit growth and development. These sources are primarily used to increase photosynthetic photon flux levels, certain phytochemicals, for examples carotenoids in tomato; however, these lights also emit some unnecessary wavelengths, which are not known to promote growth of fruit and vegetables, as these wavelengths are outside the photosynthetically active radiation (PAR) spectrum (Kim et al., 2004d). Light-emitting diodes (LEDs) represent a relatively new technology for the greenhouse industry, as they emit light of narrow bandwidths. These diodes are compact, give high radiance and are very easy to integrate into electronic systems; LEDs have unique properties, which allow for high luminous intensity, radiant intensity and for a convenient manipulation of the light spectrum (Branas et al., 2013).

Usage of LEDs has become more feasible as a form of light source, because LEDs have unique properties (energy-efficient and long-lasting). These lights are affordable and they do not contain unnecessary, low quality wavelengths. Therefore, LEDs can be employed to promote growth of fruit and vegetables in agriculture, particularly in horticulture, as they aid in plant development. Further, LEDs are easily controllable light sources and their use can improve the nutritional content of certain commodities, such as lettuce, pepper, strawberry and radish, while improving or maintaining yield and giving high quality produce (Morrow, 2008; Yeh and Chung, 2009; Mitchell et al., 2012). From a research perspective, LEDs have the advantage of being able to emit a small bandwidth and, because of the small amount of heat they emit, it is possible to separate the heat effect of a light source from the actual light effect. As a result, they are used as light sources on postharvest preservation of plants (Buchert et al., 2011). Moreover, in the food industry, food safety is of major concern during fruit production and storage, food processing, manufacturing and retail (supermarket and meat shops) establishments are shifting from traditional fixtures, such as incandescent and fluorescent lamps, to light emitting diode (LED) products. It is important to consider that such equipment can be used to meet strict food safety guidelines during application. In the medical field the use of therapeutic LEDs has also been successful (Kessler et al., 2001), so producers of fresh fruit and vegetables were assured of the food safety of LED, meaning, technologies developed to keep food safe can employ LED technologies.

The quality of light, *i.e*., the colour reaching the surface of the plant (Johkan et al., 2010), strongly influences plant development. The major sources of light or energy influencing plants are blue and red light, affecting plant growth and being sources of energy for photosynthetic CO_2 assimilation. In blue or red light, the action spectra have maxima between 400 to 800 nm (Cosgrove, 1981; Kasajima et al., 2008). The beneficial effects of combining red and blue light for illuminating plants were proven by Brown et al. (1995). These authors had evaluated the effect of LEDs on growth, dry matter partitioning and carotenoids of `Hungarian Wax' pepper *(Capsicum annum* L.) after these plants had been grown under red LEDs only compared with plants grown under red LEDs with supplemental blue or far-red radiation or under broad spectrum metal halide (MH) lamps. Brown et al. (1995) reported that red (660 nm) and blue (550 nm) LEDs may be suitable, in proper combination with other wavelengths of light, for the culture of pepper plants in tightly controlled environments.

A study by Lee et al. (2007) demonstrated that supplemental light quality can be strategically used to enhance the nutritional value and growth of lettuce plants grown under red-blue-white (RBW) LED lights. Others studies were performed by various authors to demonstrate the beneficial effects of blue and red LEDs on various fruits and vegetables grown in a controlled environment (Yanagi et al., 1996; Tanaka et al., 1998; Yorio et al., 2001; Hanyu and Shoji, 2002; Lian et al., 2002; Nhut et al., 2003; Dougher and Bugbee, 2004; Kim et al., 2004b; Shin et al., 2008). The most effective light sources for greenhouse tomato and lettuce production are red light (RL) (650-750 nm) and blue light (BL) (450-490 nm).

Yield, growth of tissues and cells, photosynthesis and concentration of phytochemicals is influenced by the quality of light (Liu, 1993). At present, studies concerning light treatment have been focused on how radiation of UV-C, HPS, inflorescence and LEDs (even though they are still gaining popularity) affect physiology and morphology of plants (Holzinger and Lutz, 2006; Poppe et al., 2002; Zancana et al., 2008). In the leaves of various plants the ultrastructure of organelles were evaluated following diverse treatments with blue, red, and far-red light.

There is only little information concerning illumination of tomatoes by supplemental lighting. Lu et al. (2012) concluded that white and red LEDs resulted in higher yield of fresh market tomato than HPS and yellow LEDs. Several studies have been undertaken to find how blue light affects quality and growth of tomatoes. A study by Hernandes and Kubota (2012) revealed that

during early stages of development of tomato seedlings, blue light had no positive effect on growth of the fruit; however, the physiology of the plant was not analysed.

The aim of this review is to describe the effects of LEDs and other light sources on developmental stages of horticultural commodities and to also to examine the properties of supplemental light to establish the current knowledge base, while pointing out potential gaps that need to be closed and applications that could be developed to produce more/ better quality tomatoes.

2.2 ORIGIN AND HISTORY OF TOMATO

Central America is a region where tomato originated and was first cultivated. In Mexico the first selections were made. After 1535, the tomato was brought to Europe by Spaniards and then, shortly before 1604, it was introduced to the East by the Portuguese. The Portuguese also took the plant to their territories around southern Africa at an early date, around 1850. Tomato was brought from eastern Africa to the Cape. In Afrikaans the tomato is called “tamatie”, this probably originated from the Malay word “tamatte” (Bai and Lindhout, 2007). Today tomato is one of the most important vegetable crops in the entire world; that brings Joseph B. Feldt’s prophesy of 1845 to fulfilment: “Like the potato slow in its rise, it is likely to be slow in its fall.”

The first greenhouse that was used to grow tomato was in 1932 (Went, 1944). Greenhouse production constitutes a major part of commercial horticultural production. A rapid increase in the use of greenhouses for tomato production tomatoes has been observed worldwide in the last 30 years (Jones, 2007).

2.3 PROPERTIES OF LEDs

2.3.1 Overview of LED technology

An LED produces light though electroluminescence, which consists of positive (p-type) and negative (n-type) junction. When an LED is connected to a power source, a flow of currents starts from the p-type junction to n-type junction, resulting in the flow of electrons. During this process, electrons are able to recombine with electron-holes, causing the electrons to fall to a lower energy level and thereby releasing photons. This process is termed electroluminescence (Gupta and Jatothu, 2013).

The band gap energy of the semiconductor material determines the colour of light emitted. Red and infrared light use gallium arsenide, while orange, green, red and yellow use indium gallium aluminum phosphide and blue light uses gallium nitride (Yeh and Chung, 2009; Gupta and Jatothu, 2013). Ultra violet radiation is emitted by LEDs composed of indium gallium nitride, which have a wavelength of about 210 nm (Shur and Gaska, 2010). White LEDs have been produced or created by combining UV-LED and tri-colour phosphor coating or by combining yellow phosphor with blue LED (Park et al., 2014). Otherwise the combination of green, red and blue LEDs can also produce white light (Denbaars, 2013).

Significant quantities of visible light energy can be produced by LEDs in terms of lumens per unit input of electrical power ($\mu mol/m^2\ s^{-1}$), and consequently result in a very high luminous efficacy. The United States Department of Energy determined that there is a similarity between the current luminous efficacy of LED, fluorescent and high intensity discharge luminaries. Likewise, the number of photons for LEDs is similar to HPS lamps according to Nelson and Bugbee. (2014); LED performance can be evaluated by measuring electrical efficiency. The electrical efficacy (the ratio of luminous flux to power) of HPS lamps is approximately the same as for LEDs, but it is higher than that of fluorescent lamps (Pinho et al., 2012).

Electrical efficiency of LEDs also varies with wavelength. An electrical efficiency of above 60% has been reached using blue LEDs. In contrast, an estimate of about 10% of the electrical efficiency have been reached using UV LEDs (Dobrinsky et al., 2012). A peak emission of about 275 nm reaches the maximum electrical efficiency of UV LEDs of 8%, while medium pressure

mercury lamps reach a maximum of about 8% of electrical efficiency. These LEDs emit UV radiation within the range of 200 to 300 nm (Ibrahim et al., 2014). When improving the luminous efficiency, light extraction efficiency must be improved; this can be achieved by lowering the internal reflection within the chip (Zhmakin, 2011; Dobrinsky et al., 2012). Hence, the predictions of efficiency improvement are advantageous and compare favourably with existing lighting technologies.

Light emitting diodes (Fig. 2.1) have many advantages over other light sources; they emit low radiant heat which lowers the harmful effects of radiant heat on the quality of agricultural commodities (Morrow, 2008; Mitchell et al., 2012). Nonetheless, at p-type and n-type junctions, a considerable amount of radiant heat is produced. Consequently, the use of fans and other cooling devices is necessary in unventilated storage compartments (United States Department of Energy, 2012). As LEDs can last between 50000 to 100000 h, they exceed the lifespan of conventional lighting systems, which typically last approximately 15000 h. Additionally, LEDs are compact, which helps when designing lighting systems where space is limited (Gupta and Jatothu, 2013).

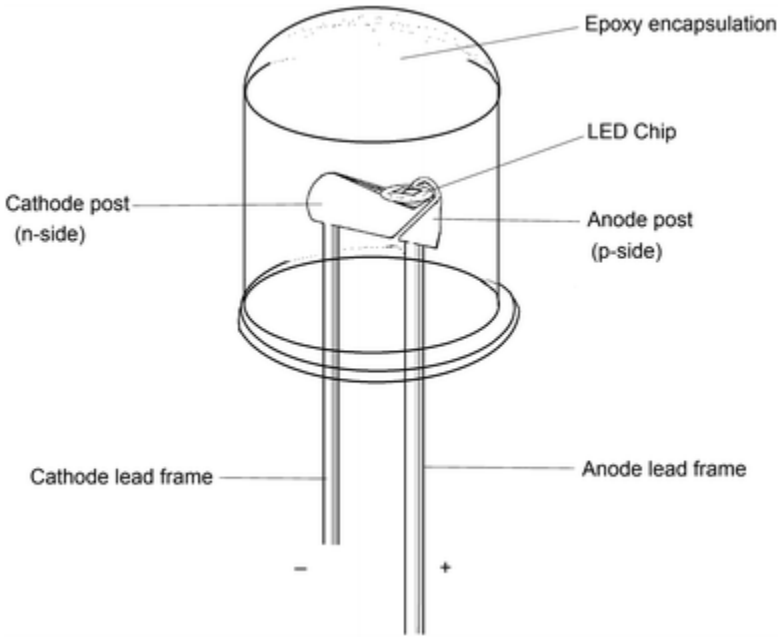

Figure 2.1: The basic structure of a light emitting diode (LED)

(https://www.merg.org.uk/merg_resources/led.php) (Accessed 20 June 2017)

2.4 ADVANTAGES OF LEDs – Low radiation heat production

The use of high intensity discharge (HID) lamps in horticultural enterprises have been recommended by growers as it provides additional lighting for plant growth and development. High intensity discharge (HID) also emit long wave radiation, causing the surface of the plant to get heated; however, a minimal long wave radiation is produced by LEDs (Mitchell et al., 2012). For small scale horticultural application, LEDs can, therefore, be placed closer to small crops or be directed onto certain parts of plants, a feature HID lamps cannot provide. The use of this feature allows for the prediction of intra-canopy lighting. To supply the upper part of the plants with light, LEDs are typically placed between the canopies of plants; however, according to data that was collected on tomatoes and cucumbers, such application has limited success (Gislerod et al., 2012). When blue and red light were used to supplement HPS lamps and plants were treated with intra-canopy lighting no significant increase in cucumber cumulative fresh mass was observed in the fruit (Trouwborst et al., 2010; Hao et al., 2012). More noticeably, for the tomato plant, a higher energy efficiency was seen, meaning that there is potential for energy savings because they can provide the 'useful' wavelengths without wasting energy for production of the long wavelength radiation.

2.5 ENHANCING THE NUTRITIONAL QUALITY OF PLANTS THROUGH LEDs

When plants are subjected to light, production of various nutrients and secondary metabolites, particularly those with antioxidant function is stimulated; these compounds are part of a defence mechanism against light stress and the resultant excess of reactive oxidation species (Darko et al. 2014). In a review by Bian (2015), the effects of light intensity, quality of LEDs and photoperiod on the accumulation of nutrients in different vegetable crops in a greenhouse was reviewed. It was concluded that in crops such as lettuce and tomato (Li and Kubota, 2009; Samuoliene et al., 2012a, 2013), Chinese cabbage (Avercheva et al., 2014) and pea seedlings (Wu et al., 2007), various LED light treatment resulted in an increase in the concentration of antioxidants and other bioactive compounds in the treated crop. The use of LEDs can also

enhance the nutritional quality of fruit. Grapefruit irradiated with blue and red light (450 and 660 nm) at 50 μmol m^{-2} s^{-1} for 3 h before and after sunset and before sunrise had higher sugar and anthocyanin concentrations compared with the control that did not receive supplemental light (Kondo et al., 2014). Up to now it is not fully understood how light enhances the nutritional content of some fruit and vegetables. It would, however, be useful to be able to change the spectral composition of light so as to manipulate growth and development of fruit through a certain optimal light spectrum supplied to fruit and vegetables. This would help food producers to manipulate the lighting routines so as to accelerate growth, and particularly quality, of food stuff.

2.6 TYPES OF LED LIGHTS

The light under which plants are grown (Fig. 2.2) affects growth and development in a complex manner. Light quality and quantity initiate a signalling response in specific photoreceptors, such as phytochrome, cryptochrome and phototropin, which alter the expression of a large number of genes (Casal, 2000). While specific responses of plants to a certain light spectrum may sometimes be predictable, the overall plant response is generally difficult to predict due to the complicated interaction of many different responses (Hogewoning et al., 2010). Light emitting diodes, which are characterized by relatively narrow-band spectra, are employed to trigger specific plant responses to certain light quality.

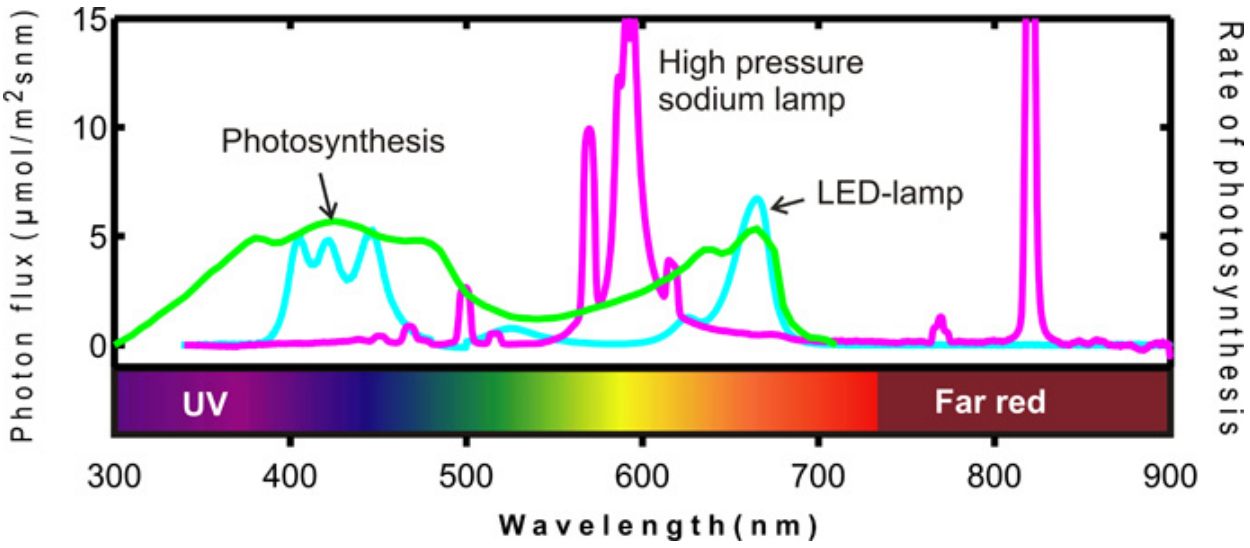

Figure 2.2: Wavelength of light emitting diodes and high pressure sodium lamps in relation to wavelength used by photosynthesis

(https://3c1703fe8d.site.internapcdn.net/newman/gfx/news/hires/2013/1-plantscommun.jp) (Accessed 17 July 2017)

2.6.1 The importance of red and blue light

Plants have photoreceptors, which are proteins that are specially designed to perceive light and signal certain biological effects in the plant. The effect of light on plant morphology is called photomorphogenesis. Plants have blue (440-500 nm) and red light (600-700 nm) photoreceptors, which absorb at various wavelengths. There are two features of red light that make red LEDs to be used widely. Firstly, plant pigments efficiently absorb light in the red wavelengths (600 to 700 nm), as visible in the McCree curves (Figure 2.3) (Sager and McFarlane, 1997); secondly, early LEDs were red with the most efficient emitting at 660 nm, close to an absorption peak of chlorophyll. The other main wavelengths included in early studies has been in the blue region (400 to 500 nm) of visible light. In plants, blue light plays a significant role, regulating phototropism (Blaauw and Blaauw-Jansen, 1970) and controlling stomatal movement (Schwartz and Zeiger, 1984). Hoenecke et al. (1992) demonstrated the need of supplementing high output red LEDs with some blue light to improve growth and yield. The authors also discovered that wheat exposed to red LEDs without the supplementation of blue light, plants failed to synthesize chlorophyll. Supplementation of red LEDs with 30 mmol m^{-2} s^{-1} blue light was able to restore chlorophyll synthase activity. An increase in photosynthetic photon flux (PPF) from 11 to 64

mmol m^{-2} s^{-1} resulted in an increase in chlorophyll synthase of potatoes grown *in vitro* under red LED; however, an increase in shoot length was observed in plants treated with red LED (Miyashita et al., 1994).

In a study by Yanagi et al. (1996) on lettuce plants it was concluded that red LEDs alone resulted in an increase in leaf number and in longer stems compared with plants subjected to blue LEDs only. Goins et al. (1997) demonstrated that wheat treated with red LEDs could complete its lifecycle, while if red LEDs were supplemented with blue light, larger plants were produced with larger leaves. Yorio et al. (1998) studied the effect of the intensity of blue LED light and reported that the photosynthetic rate and shoot dry matter increased with an increase in intensity of blue light. In the same study, the yield of tomato, spinach, lettuce and radish was reduced when these commodities were grown under red LED only, but when the red LEDs were supplemented with 35 mmol $m^{-2}s^{-1}$ blue fluorescence, yields increased. These studies clearly demonstrated that supplying a combination of red and blue LEDs to most agricultural commodities gives higher yields of plants under protected cultivation.

Blue light has many positive effects, amongst them the activation of the cryptochromes, a class of flavoproteins that are sensitive to blue light, and match the absorption spectra of carotenoids and chlorophyll as demonstrated on the morphology of green vegetables, photosynthesis and growth (Yanagi et al., 1996). In cabbage plants, high leaf chlorophyll concentration is caused by blue LEDs (440−476 nm) individually or in combination with red LEDs (Mizuno et al., 2011, Li et al., 2012); the same red-blue combination can also stimulate biomass accumulation in cabbage plants (Li et al., 2012) and lettuce (Johkan et al., 2010). When red LEDs were supplemented with blue light from blue fluorescent lamps, similar results were obtained, the biomass of treated vegetables was increased (Yorio et al., 2001; Yorio et al., 2011). The use of supplemental blue light has different effects on green vegetables, affecting leaf coloration (Stutte et al., 2009; Mizuno et al., 2011), increasing leaf polyphenol (Johkan et al., 2010), carotenoid (Lefsrud et al., 2008; Li and Kubota, 2009) and anthocyanin concentrations (Li and Kubota, 2009; Stutte et al., 2009).

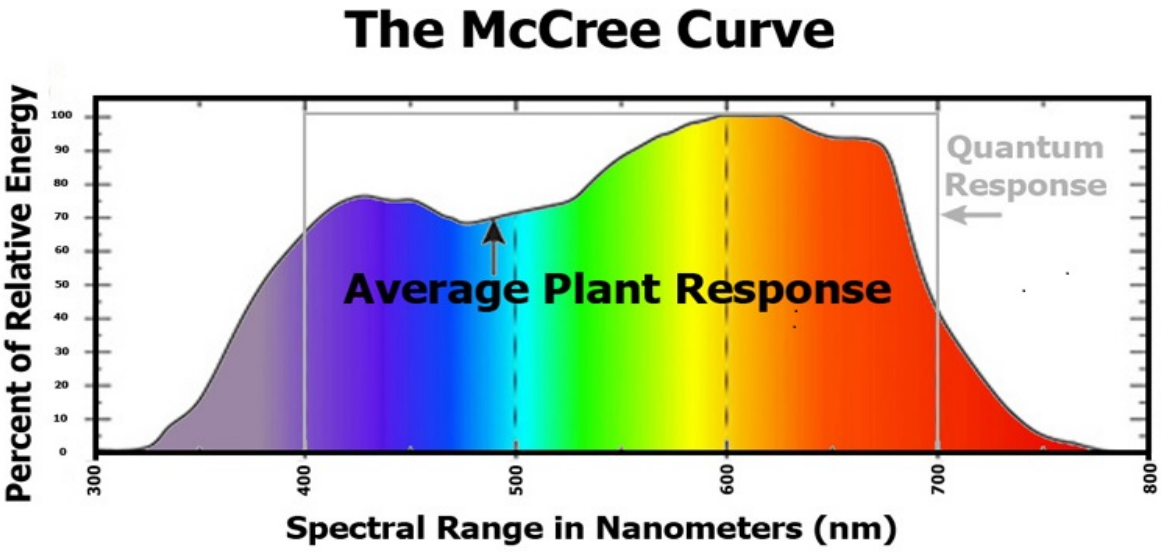

Figure 2.3: The McCree curve

https://smartgrow.systems/plant-light-dli-calculator/ (Accessed 21 November 2017)

2.6.2 Effect of far-red and infrared LEDs on plants

Leaf anatomy can change following treatment with different light combinations, as reported for *Capsicum* pepper by Schuerger et al. (1997). Red (660 nm) LEDs combined with far red (735 nm) LEDs resulted in alterations in leaf thickness and chloroplast number per cell, while when supplemented with blue LEDs leaf thickness, examined as the leaf cross sectional area, was reduced in plants that did not receive blue light, whereas an intermediate response was observed under red light. Blue light treated plants had the thickest leaves with the highest number of chloroplast per cell (Schuerger et al., 1997). Kim et al. (2005) discussed different studies using far red (FR) light and found that the addition of 24% green light (500–600 nm) to red and blue LEDs enhanced the growth of lettuce and tomato plants compared with plants grown under cool white fluorescent lamps.

In a study by Johnson et al. (1996) the effect of infrared LEDs on etiolated oat seedlings using LEDs emitting at 880 nm and 935 nm was examined. Seedlings grown with 880 nm LEDs were

shorter compared with seedlings grown with dark or 935 nm, but a faster leaf emergence was observed. A lower proportion of coleoptile to seed tissue was observed in seedlings grown with infrared LEDs, whereas a higher proportion of mesocotyl to coleoptile tissue was observed. It was also observed that seedlings grown with infrared LEDs grew straight.

Stutte et al. (2009) demonstrated that far-red LED light (700 and 725 nm) is too far outside the PAR range to support adequate photosynthesis and growth of lettuce. When far red was combined with red LEDs or white fluorescent light, however, several growth characteristics were affected, such as increased leaf length and biomass, while anthocyanin, chlorophyll and carotenoid concentrations were affected negatively (Li and Kubota 2009; Stutte et al., 2009). Growth promotion under far red light supplemented with red LEDs has been associated with improved light interception due to an increase in leaf area (Kubota et al., 2011).

As red light forms the basis of radiation necessary for growth and development, as well as for photosynthesis in plants, this light is usually the basal component in lighting spectra. Various wavelengths of red light, however, may have varying effects on plants. Goins et al. (2001) evaluated the effect of various wavelengths and found an increase in biomass and, therefore, yield in lettuce as the wavelength was increased from 660 to 690 nm in 10 nm intervals. For cultivation of green vegetables, the use of red LEDs at 640 nm seems optimal (Lefsrud et al., 2008; Žukauskas et al., 2011; Samuolienė et al., 2012a; Samuolienė et al., 2012b). Illumination with approximately 660 nm can also be beneficial to plant growth (Brazaitytė et al., 2006; Li and Kubota, 2009; Mizuno et al., 2011; Tarakanov et al., 2012). The use of red light individually, or in combination with natural illumination or with fluorescent lights, had no significant effect on growth parameters of leafy vegetables, but increased leaf antioxidant concentration. Red LED light at 660 nm stimulated the accumulation of anthocyanins in red cabbage grown under controlled environment (Mizuno et al., 2011). When leaf lettuce plants were grown under red light at 658 nm supplemented with white fluorescent lamps, phenolics concentrations increased by 6% (Li and Kubota, 2009). Red LEDs (640 nm) used to illuminate cabbages under controlled environment enhanced lutein accumulation when applied as a short term pre-harvest treatment (Lefsrud et al., 2008). Various experiments were performed on botanical varieties of lettuce with 638 nm LED light (supplemented with HPS and natural illumination) as pre-harvest treatment. An increase in leaf antioxidant concentration, tocopherol and phenolic compounds was observed

following this illumination (Žukauskas et al., 2011; Samuolienė et al., 2012a). Similarly, Bliznikas et al. (2012) reported an increase in vitamin C and carbohydrate concentration in other leafy vegetables cultivated under red (638 nm) LEDs.

2.6.3 Effect of green light on plants

Previous studies have established that, even when blue light is added to red light, white light still results in better plant growth than the red/blue combination. Indeed, to human beings, plants appear purplish grey when grown under blue plus red light and it is very difficult to diagnose disease (Fig. 2.4A). The addition of small amounts of green light is a possible solution to this problem (Fig. 2.4B). Kim et al. (2004a) conducted a study growing lettuce plants under blue and red LED light with and without green light using LEDs at the same total PPF. No impact on lettuce growth was observed for all measurable characteristics (leaf area, photosynthesis rate, and shoot mass and leaf number) with and without green light. In a further study, Kim et al. (2004b) determined the effects of green light supplied at high level on lettuce under a total PPF of about 150 mmol $m^{-2}s^{-1}$ and a photoperiod of about 18 hours. Red and green light with and without green fluorescence (GF) was used in the study. Lettuce plants illuminated with RGB had a larger leaf area and higher fresh and dry mass compared with lettuce grown under RB alone. All lettuce plants grown under GF had a lower biomass in comparison with other treatments.

Kim et al. (2006), concluding on experiments carried out with the supplementing of GF to red and blue light, described a reduction in plant growth when more than 50% green light was used, whereas combinations including up to 24% green light enhanced growth for lettuce species.

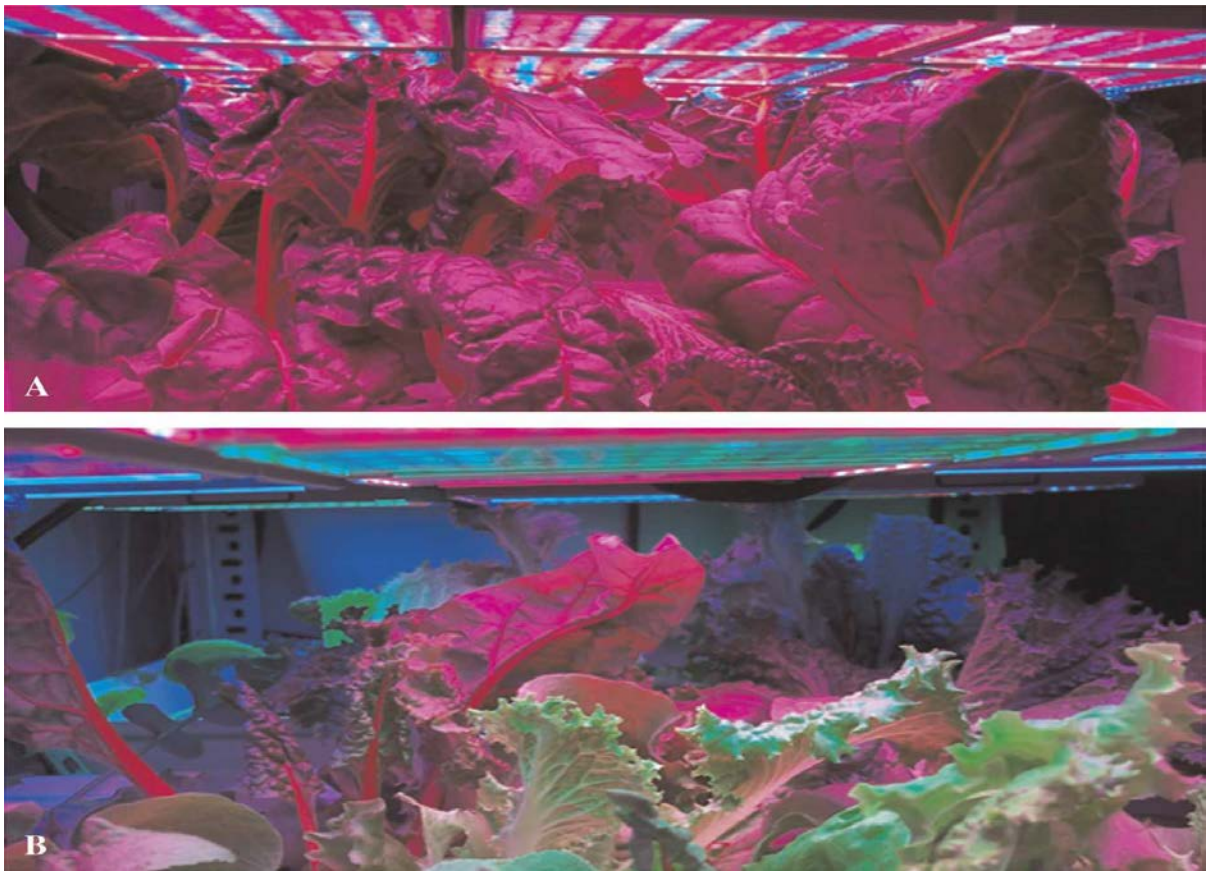

Figure 2.4: Swiss chard and lettuce plants illuminated with red combined with blue (A) or red combined with blue plus green (B) LEDs (Folta and Maruhnich, 2007)

Growth rate of the plants was normal under both combinations, but under red combined with blue light (A), leaves appeared purplish, making visual assessment of plant condition difficult. When red plus blue was supplemented with green, the problem for human visual perception was resolved (Folta and Maruhnich, 2007).

Folta and Maruhnich (2007) conducted various studies on the effect of green light on agricultural commodities and showed that Swiss chard and lettuce plants developed abnormal intumescence (small, bump-like protrusions on the surface of leaves, petioles and stems; (https://ag.umass.edu/greenhouse-floriculture/fact-sheets/oedema-intumescences) on older leaves (Fig. 2.5 A).

Figure 2.5: Abaxial edema in a fully expanded cowpea leaf grown under less than 10% blue light-emitting diode (LED) light (A) and terminal edema in 'Triton' pepper with intumescent growths forming on the shoot apex as well as other growths occurring on flower sepal and mature and immature leaves grown at 15% blue LED light (B) (Folta and Maruhnich, 2007)

This physiological disorder did not develop when plants were illuminated with high blue light. Severe occurrence of foliar edema, an abnormal intumescence, was also observed on pepper plants illuminated with blue plus red LEDs. Extensive edema was observed on both flower buds and leaves, even though fruit set occurred, the edema inhibited photosynthetic productivity (Fig. 2.5B). Increasing the percentage of blue light did not mitigate the disorder on pepper. The use of additional UV-A (330-365 nm) ''black lights'' was inconclusive in the preliminary analysis, most likely a result of the low energy flux from such lamps and the unequal distances of the UV-A source to the photosynthetic surfaces of the plants. Tomatoes, grown under the same UV-A LED lamps were not affected by edema, indicating that even within the solanaceous species, different susceptibilities to this physiological disorder exist (Williams et al., 2016).

The use of green light on vegetable crops has certain valuable physiological effects. Green fluorescent light (GF), as well as green 510, 520, 530 nm LED lights supplemented with blue and red LEDs resulted in increased growth of lettuce (Kim et al., 2004; Johkan et al., 2012). Green LEDs at 505, 530 and 535 nm supplemental to HPS lighting had a positive effect on quality attributes of different varieties of greenhouse-grown lettuce (Samuolienė et al., 2012b, Samuolienė et al., 2012d), increased ascorbic acid, and tocopherol concentrations.

2.7 LAMP PLACEMENT TO INCREASE LIGHTING EFFICIENCY

Light can be of high quality and give high yield, but there are other factors in relation to plant growth that need to be taken into account, such as the position of light sources. The radiation energy that is emitted from the source of light onto the surface of a plant is related to the inverse square of the distance between plant surface and light source (Bickford and Dunn, 1972). If the distance from the light source to the surface of the plant or plant part is reduced, a large impact on the incident light level will occur. Cooler LEDs can be brought closer to the tissues of the plant in comparison with HID lamps. As a result, LEDs can give the same incident PPF as HIDs, even when they are operated at a lower energy level.

Purdue University and Orbital Technologies Corporation developed a reconfigurable LED lighting array which help minimize electrical inputs for crop lighting. Studies by Massa et al. (2005a; 2005b) described a lighting arrangement made of 16 lightsicles (individual units of the lighting array), with each lightsicles made of 20 ‘‘light engines’’ with numerous printed circuit of LEDs. Columns of red and blue LEDs are found in each square light engine, these are independently current-controlled as to allow colour blending and continuous dimming. The arrangements of lightsicles is vertical, separate, intra canopy configuration, whereby a crop stand of planophile plants (having the leaves more or less horizontal), such as tomatoes can grow up around and surround the vertical light strips. The LED light engines are energized individually from the bottom up to keep pace with the top of the growing crop canopy (Massa et al., 2005b).

2.8 EFFECT OF LIGHT QUALITY ON PLANT GROWTH AND DEVELOPMENT

2.8.1 Seed dormancy and germination

The release of seed dormancy and subsequent germination is conditioned by light, a critical environmental factor for this developmental period. This process can, however, be affected by light quality. A range of responses is exhibited by different plants to blue and green light with regard to dormancy release (Goggin and Steadman, 2012). In some plants, seed dormancy is induced by darkness combined with stratification. It is very difficult for light to stimulate some dormant seeds, unless the seed is treated with a 20-d dark stratification before being exposed to sunlight. Dormancy can be maintained in seeds that are stratified in blue light, irrespective of the presence or absence of far-red light. Interestingly, green light acts the same way as blue light to inhibit dormancy release (Goggin et al., 2008).

The light conditions during seed maturation has an effect on the germination rate. Generally, a lower germination rate is observed in seeds that are allowed to mature in a shaded environment (low red/far-red ratio) than those seeds that have matured in an environment with high red or far red light (Dechaine et al., 2009).

Seed matured in the shade (dense canopy or under covers) may avoid adverse germination conditions by maintaining dormancy. The fact that green light is enriched in a shade environment relative to red and blue light, can stimulate responses associated with shade avoidance. It has, therefore, been hypothesized in some species, that green light can serve as a regulator of the germination of the seeds. This is also consistent with this interpretation that green light overcomes dormancy.

2.8.2 Seedling establishment

Just after germination, a seedling of dicotyledonous plants adjusts the elongation of its hypocotyl to best adapt to the prevailing light conditions. The elongation of the hypo- or epicotyl decreases significantly after the seedling has emerged from the soil and is exposed to light. In hypogeal germination, this light perception is accompanied by the opening of the hook and the expression of genes which support the formation of photosynthetic structures (Motsa et al., 2015). Under

high fluence-rate, blue light inhibits stem elongation (Folta and Spalding, 2001; Ahmad et al., 2002). This effect is primarily mediated by cryptochrome receptors and continues, as long as the blue light is present. This hypocotyl elongation decreases, if red and far red light is supplied to the seedling, a stimulus perceived through phytochrome A and B. Time lapse image analysis has revealed the exact timing of early changes in the rate of elongation, showing that blue, red, and far-red responses occur within minutes of illumination with such lights. These light responses are mediated by cryptochrome and phytochrome receptors (Parks et al., 2001).

Folta (2004) reported that illumination of seedlings does not decrease their growth rate. Instead, growth of the seedlings is faster than under dark conditions, at times approaching 150% of the etiolated pace. Within minutes of illumination, the same author (Folta, 2004) recorded a response to green light and the growth rate was reversed to the one in the dark, when the light was toggled. In all photoreceptor mutant backgrounds, the response continued; this suggests that the response either was of numerous photoreceptor classes to dim green light or facilitated by a not yet discovered receptor. It was challenging to determine how green light could activate a known set of light sensors, yet drive responses that were diametric to normal light activation. Another study was conducted and results showed that seedlings which were allowed to grow under dim (<4 µmol $m^{-2}s^{-1}$) blue and red light supplemented with green light (530 nm) were much taller than those seedlings grown under blue or red light alone (Cocetta et al., 2017).

A report by Bouly et al. (2007) showed a mechanism for this phenomenon in greater details. *Arabidopsis* seedlings were grown under white or blue light (420-650 nm) supplemented with green-yellow light (563 nm). Red or far red light supplemented with green light resulted in a diminished red light response within an hour. Wang et al. (2012) reported that seedlings grown under red plus blue and green light were longer than those grown without the green light supplement.

This green light counteraction was further examined in photoreceptor mutants, where it was then suggested that the blue light was perceived by cryptochrome (Bouly et al., 2007; Sellaro et al., 2010). The strongest reactions to green light were observed under low light conditions, a conclusion inconsistent with Sellaro et al. (2010) who examined different ratios of blue: green light. These authors indicated that reducing the blue to green light ratio, results in an increase in

hypocotyl length over a broad range of fluence-rates (Sellaro et al., 2010). Simultaneous blue and green light irradiation did not reverse the response of cryptochrome-mediated stem growth inhibition (Wang et al., 2009). Increasing the magnitude of the blue light was a result of additional green light, indicating that high fluence-rate blue light supersedes green-light-induced growth promotion, again demonstrating green light effects to be low-light effects.

2.8.3 Vegetative growth

Klein and coworkers (Klein, 1964; Klein et al., 1965) performed various studies in the 1960s on the effects of light on plant growth of Marigold plants (*Tagetes*) and tomato plants using near ultraviolet and green light, elucidating that the growth rate of various organisms, including fungi, algae, and cell cultures is affected by green wavelengths. In 1957 Went had already demonstrated that tomato seedlings reach higher dry mass when subjected to reduced green lights compared to white light. Dougher and Bugbee (2001) demonstrated that the dry mass of lettuce was reduced when grown under yellow light (580–600 nm). Lettuce plants were grown in six light treatments comprising five light fractions of 0, 2, 6% from high-pressure sodium lamps and 6, 12, 26% from metal halide lamps, high-pressure sodium lamps (HPS) and metal halide (MH) of 6% blue affected plant growth significantly. Scientists from NASA conducted various studies on plant growth in the presence of various lights. Lettuce was found to have higher fresh and dry mass of shoots, and larger leaves, when grown in a combination of red, blue and green LEDs than those grown exclusively under red or blue (Kim et al., 2004a,b). These results demonstrate that while blue and red light promote photosynthesis effectively, green light is able to penetrate plant leaves more efficiently. An advantage is, therefore, gained from additional green light illumination, resulting in an increased carbon fixation under green light in spinach (Sun et al., 1998) and lettuce (Kim et al., 2004b). There was no significant difference between lettuce grown under white fluorescent lamps (17% yellow light) and under red-blue (0% yellow light) light. These results contradict results by Dougher and Bugbee (2001) who reported lettuce leaf area, dry mass, chlorophyll concentration and specific leaf area to be significantly higher when grown under high pressure sodium lamps (HPS) and metal halide (MH) lamps, suggesting wavelengths other than blue and red also affect plant growth. Kim et al. (2004a) suggested that this discrepancy might be due to the different lettuce cultivars, as well as differences in light intensity and quality used in the experiments.

2.8.4 Flowering

Light influences the transition from vegetative growth to floral development (Guo et al., 1998; Mouradov et al., 2002).Various wavelengths exhibit certain roles in the regulation of floral initiation. Red light slows down floral initiation via the phytochrome *phy* B receptor, whereas induction is accelerated by blue light through the cryptochrome *cry2* receptor (Guo et al., 1998; Valverde et al., 2004). Following blue light treatment, green light may inactivate the *cry2* receptor, if the same mechanism is functioning later in the development. Banerjee et al. (2007) tested this hypothesis by adding green light to ambient light conditions and found that it delays the time required for blue light-treated plants to flower. Consistent with this outcome, the cry2-mediated induction of flowering locus T (FT) transcript levels was also reversed by simultaneous irradiation with green LED light; the effects of green LED light were not observed in the cry2 mutant background (Banerjee et al., 2007). The heading time of some plants does not seem to be influenced by blue LEDs; however, wheat plants grown under green-yellow light with a very high fluence-rate only needed several days to reach 50% heading (Kasajima et al., 2007). When analysing individual wavebands, 540 nm significantly stimulated flowering (Kasajima et al., 2008; 2009).

2.9 EFFECT OF LIGHT QUALITY ON PHYTOCHEMICAL COMPOSITION OF PLANTS, FRUIT AND VEGETABLES

Human health can be maintained by vegetables because they can produce high concentrations of beneficial phytochemicals, such as vitamins, soluble sugars, soluble proteins, carotenoids and secondary antioxidants. Many studies have shown that phytochemical accumulation in vegetables is significantly affected by light conditions, environmental temperature and genotype (Tiwari et al., 2013).

2.9.1 BENEFICIAL SUBSTANCES

2.9.1.1 Anthocyanins

Anthocyanins are common pigments in plants; their importance for humans lies in their powerful antioxidant activity and resultant health benefits (Youdim et al., 2002). The level of anthocyanin in plant foliage can be increased by environmental stresses, such as insufficient light, nutritional deficiency and low temperature; however, the effects of these stresses differ between various plant species.

Gene expression is mostly induced by blue light (400–500 nm); light of this quality induces the synthesis of anthocyanins through the activation of gene encoding *cry 1* (Ahmad et al., 1995). The accumulation of anthocyanins in the presence of light depends on the fluence-rate (Lin et al., 1996). Blue light can be used to regulate anthocyanin biosynthesis in many plant species, such as lettuce (*Lactuca sativa L*), tomato (*Solanum lycopersicum*), and rapeseed (*Brassica napus*) (Giliberto et al., 2005; Zhang and Folta, 2012) through various cryptochrome receptors in higher plants (Cashmore et al., 1999). Consequently, the anthocyanin content in tomato fruit can be increased by blue light (Giliberto et al., 2005). The degree of anthocyanin decrease relies on the fluence-rate of green light delivered with blue light (Zhang and Folta, 2012). A very close examination of this response in *Arabidopsis cryptochrome1* mutants revealed that this is cryptochrome-dependent green light response (Bouly et al., 2007). The results from this experiments pointed to a green light effect discrepancy or paradox, because an increase in visible light leads to a decrease in the magnitude of the green light-driven responses. The most effective wavelengths to increase anthocyanin accumulation and biosynthesis are red, blue and UV-A light, whereas far-red light has a negative effect on anthocyanin accumulation in leafy vegetables.

2.9.1.2 Soluble proteins and soluble sugars

Vegetables contain soluble proteins and sugars, which are vital nutritional components that provide energy and essential proteins needed by the human body to function properly. The quality of light significantly affects accumulation and biosynthesis of sugars and soluble proteins. According to previous studies, soluble sugars in cucumber, tomato and radish can be increased by red LED light (Cui et al., 2009; Zhang et al., 2009; Zhang et al., 2010). Zhang et

al. (2009) demonstrated that red light can increase sugar in fruit concentrations and restrict soluble protein biosynthesis in pea seedlings. Chang et al. (2010) found an increase in soluble sugars in tomato seedlings when treated with blue LED lights compared with any other types of LED light; however, when red and blue LED lights were combined, even higher amounts of soluble proteins were observed. Zhang et al. (2010) also reported similar results; radish seedlings irradiated with blue light or a combination of red and blue LED lights had a higher concentration of soluble proteins than seedlings irradiated with only red or white LED. Furthermore, Lin et al. (2013) found that a combination of red, blue and white LED light used as a supplementary light increased the soluble sugar level of hydroponically grown lettuce.

Taken together, plant sugar and soluble protein levels can be influenced by light quality, but the effect is cultivar- and species-dependent. The combination of red and blue light increases sugars and proteins in fruit and vegetables more effectively. This may be due to red and blue light being the two major types of light driving the biosynthesis of photosynthates and the biosynthesis of proteins being facilitated by blue light (Li and Pan, 1994).

2.9.1.3 Ascorbic acid

Ascorbic acid (AsA, Vitamin C) has various functions in plants, such as promoting cell growth, scavenging reactive oxygen species and providing a precursor for oxalate (Conklin, 2001). In humans, AsA can prevent scorbutus (scurvy) (Irwin and Hutchins, 1976); thus, to promote human health, daily uptake of 10 mg AsA (Kallner et al., 1981) is encouraged. The human body cannot synthesis AsA; therefore, eating food containing AsA on a daily basis is highly encouraged.

In plants, Vitamin C is one of the most important antioxidants, as it plays a significant role in plant stress physiology. Light quality affects biosynthesis and accumulation of AsA. Chen et al. (2011) found a higher concentration of Vitamin C in lettuce plants grown under blue LED light and a mixture of red and blue LEDs compared with red LED lights only. These results are in accordance with the results by Ohashi-Kaneko et al. (2007) who treated lettuce, spinach and tomato with different colours using fluorescent lamps. The authors found that AsA concentrations in these vegetables were significantly increased when illuminated with higher-wavelength blue spectra (Ohashi-Kaneko et al., 2007).

For vegetables grown under controlled environment, blue LEDs or a mixture of red and blue LEDs appear to be able to facilitate AsA biosynthesis and accumulation. The use of UV light, on the other hand, has a negative effect on AsA biosynthesis and accumulation in higher plants (Kovacs and Keresztes, 2002).

2.9.1.4 Carotenoids

Carotenoids are regarded as secondary metabolites, but are also one of the most important pigments in plants. In photosynthesis they are antenna pigments capturing part of the light spectrum that chlorophyll does not capture and transfer the captured light energy onto the chlorophyll molecule in the reaction centre. Additionally, carotenoids are reactive oxygen species (ROS) scavengers, thereby reducing the damage caused by ROS to chlorophylls (Landrum and Bone, 2001). There are two main groups of carotenoids, oxygenated carotenoids (xanthophylls) and the solely carbon and hydrogen-containing carotenes. Carotenoids play an important role in curing age-related eye disease (Kopsell et al., 2007), lung cancer (Gallicchio et al., 2008, Fleshman et al., 2011) and cardiovascular diseases (Meyers et al., 2013). Carotenoids can be supplied to the human body through the consumption of vegetables. Regulation of light quality can optimise the concentration of carotenoids in vegetables, especially those cultivated under controlled environments. Plants and fruit produce antioxidants in response to slight stress exposure. As carotenoids are antioxidants, exposure of plants to slight stress is likely to increase the production of antioxidants, used as 'defence compounds'. Exposing plants to higher light intensity is likely to increase the production of carotenoids as a reaction to stress, as the plant is trying to protect the chlorophyll molecules from oxidation. In previous studies, it was found that blue light has a positive effect on carotenoid accumulation in spinach (*Spinacia oleracea*) (Bian et al. 2015), while Ohashi-Kaneko et al. (2007) found that the carotenoid concentration was higher in spinach grown under blue LEDs than under white fluorescent lamps of the same PPFD (300 μmol $m^{-2}s^{-1}$). Subsequently, Bian et al. (2015) reported that blue light exposure results in an increase in lutein and β-carotene concentrations of spinach leaves. A study by Cui et al. (2009) found that the concentration of carotenoids in cucumber (*Cucumis sativus*) and tomato (*Lycopersicum esculentum*) grown in greenhouses can be increased by red or yellow LED light exposure.

In addition to visible light, exposure of vegetables to UV-B results in the activation of carotenoid biosynthesis. Accumulation of β-carotene in tomato can be increased by exposure of the fruit to UV-B before harvest (Perez et al., 2008). Similarly, mature green tomatoes illuminated with UV-B pre-harvest, showed a significant increase in β-carotene levels and a decline of approximately 56% in lutein concentration. The removal of UV-B resulted in an increase of approximately 75% in the lutein concentration in ripe-red tomato fruit following exposure to UV-B up to the turning stage and then ripened without UV-B (Becatti et al., 2009).

From these studies, it can be concluded that the effects of red and blue lights on the carotenoid concentration in plant tissues differ between species and among cultivars. It seems also evident that in comparison to other light qualities, red, blue and UV-B lights have a stronger effect on carotenoid biosynthesis and accumulation in vegetables than other wavelengths.

2.9.1.5 Phenolic compounds

Phenolic compounds function as antioxidants by directly reacting with ROS or by enhancing the production of other antioxidant compounds (Connor et al., 2005). The effects of different quality of light on the concentration of phenolic compounds, particularly flavonoids and phenolic acids, has been previously evaluated. Supplemental UV radiation of 290 to 400 nm significantly affected the phenolic concentration in tomato fruit, resulting in an increase in phenolic compounds compared with a UV supplementation of 380 to 400 nm (Luthria et al., 2006).

In addition, a study by Samuolienė et al. (2010) demonstrated that the phenolic concentration of spinach (*Spinacia oleracea*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), mustard (*Brassica*), rocket (*Eruca sativa*), and onion leaves (*Allium cepa*), green leaf and red lettuce (*Lactuca sativa*) was increased by illumination with red light (638 nm) for 16 h before harvest. The most effective light optimising the concentration of phenolic compounds is UV-B (290 - 310 nm), probably because phenolic compounds have a strong capacity for UV-B (290-310 nm) absorption, and plants can protect themselves from photo-damage by increasing the phenolic concentration (Solovchenko and Schmitz-Eiberger, 2003; Schreiner et al., 2012).

2.9.2 HARMFUL SUBSTANCES

2.9.2.1 Nitrate

Nitrate is found in all plant tissues, as it is very important for plant growth and development. Nitrate is not toxic, but when at an acid pH (2-6), it can be converted to nitrite which can result in methaemoglobinaemia (Chan, 2011) and certain forms of cancer (Cassens, 1997). Humans take up nitrates predominantly through vegetables (Amr and Hadidi, 2001). About 80% people consume nitrates per day (Zaragoza-Dorwald, 2012). Continuous consumption of vegetables containing high levels of nitrates poses a serious threat to human health. As a result, in 2002 the World Health Organisation and the Food and Agricultural Organisation suggested that the daily consumption of nitrate should not exceed 0.07 mg kg^{-1} body weight per day. Various studies have demonstrated that plant nitrate levels are affected by two main factors, namely light treatment and nitrogenous fertiliser.

Quality of light is a key factor that regulates the concentration of nitrate in plants (Deng et al., 2000). Red light can result in a decrease in nitrate concentration in plants (Lillo and Appenroth, 2001), whereas blue light can increase the nitrogen concentration (Ohashi-Kaneko et al., 2006). In radish it was shown to have no effect in reducing nitrate (Maevskaya et al., 2005); however, in a study by Qi et al. (2007) both, blue and red LED lights, were effective in reducing the nitrate concentration in spinach compared with white or yellow light. Ohashi-Kaneko et al. (2007) investigated the effects of red and blue light, individually or in combination, on lettuce and found reduced nitrate concentration in red compared with yellow and white light. Lin et al. (2013) revealed that, under the same photosynthetic photon flux density (PPFD) (210 μmol m^{-2} s^{-1}) and photoperiod (16 h), a combination of blue, red and white LED lights can reduce the concentration of nitrate compared with a mixture of red and blue LED lights in hydroponically grown *Lactuca sativa* var. *longifolia* (lettuce). In addition, nitrate levels can be reduced by modification of the red to blue light ratio. A study by Urbonaviciute et al. (2007) using leafy vegetables found that the best red to blue ratio to reduce nitrate concentration in plants was 8:1, while a ratio of 4:1 (red: blue) was found effective in decreasing nitrate levels in lettuce grown under controlled environment. In conclusion, the influence of light quality on nitrate accumulation and metabolism in plants is complex, but red and blue lights are more effective in lowering nitrate levels than yellow and white lights.

2.9.2.2 Oxalic acid

Oxalic acid is a dicarboxylic acid whose salts are widely distributed in higher plants, particularly in spinach (Santamaria et al., 1999). Oxalates occur in two forms in plants, namely, soluble and in insoluble. The soluble form can have negative impacts on human health, causing the formation of urinary stones, calcium oxalate, and reduce the uptake of important mineral nutrients (calcium and iron) (Radek and Savage, 2008). No recommendations of accepted levels of oxalates in the human diet seem to exist. Presswood et al. (2012) revealed that the oxalate concentration in plants can be decreased by removing UV-B from the light source. Qi et al. (2007) investigated the effects of different LEDs on oxalate metabolism in spinach using four fluorescent lights (red, blue, yellow and white). Yellow and white lights increased the concentration of oxalate in the leaf blade compared with the petiole; however, under red and blue LED lights the petioles had higher oxalates than the blades. Under red light the concentration of oxalates was lower than under any other light source, indicating that removal of certain leaf parts or growing spinach under red light can reduce the oxalate concentration.

2.10 EFFECT OF PHOTOPERIOD OR LENGTH OF EXPOSURE TO LEDs ON PHYTOCHEMICALS IN VEGETABLES

Photoperiod affects plant growth and development, resulting in various physiological and morphological responses of plants (Valverde et al., 2004; Bian et al., 2015). Ali et al. (2009) working on five selected vegetables (red amaranth (*Amaranthus cruentus*), green amaranth (*Amaranthus viridis*), Swiss chard (*Beta vulgaris* subsp. vulgaris), red spinach (*Amaranthus dubius*)) and reported that the highest concentration of chlorophyll, total phenolics and total antioxidants occurred in plants grown under 12 h photoperiod, but when the photoperiod was increased to 24 h, the concentration decreased. Similarly, a study by Soffe et al. (1997) demonstrated that dry mass of lettuce and spinach increased with an extension in photoperiod from 12 to 18 h.

Previous studies have investigated the effect of pre-harvest short-term continuous illumination on plant morphological and physiological characteristics. Wu et al. (2007) investigated the effect of continuous illumination on changes in quality attributes (β-carotene, chlorophyll concentrations, antioxidant capacity) in lettuce using red (625–630 nm) and blue (465–470 nm) LEDs. The data revealed that the β-carotene concentration was increased by continuous illumination with red LEDs for 96 h. In a study conducted by Wanlai et al. (2013) the nitrate concentration in crisphead lettuce declined after 72 h following short-term pre-harvest illumination with red and blue LEDs. While the concentration of AsA and soluble sugars increased after 48 h of illumination, no changes in phytochemical content was observed after further illumination. So an increase or decrease in photoperiod affect the concentration of phytochemicals in fruit and vegetables differently.

2.11 EFFECT OF LIGHT ON DISEASE AND PEST OCCURRENCE

Massa et al. (2008) predicted future trends in LED usage for plant lighting indicating that certain lighting systems could significantly reduce insects, fungi and other pathogens on certain fruit and vegetables. Not all wavelength are, however, able to reduce the ability of fungi to multiply, or of insects to feed on the host species and reproduce (Massa et al., 2008). Only little information is published on the effect of light on diseases and pests. Studies by Vanninen et al. (2010; 2012) and Johansen et al. (2011) proposed that the colour of light can induce changes in primary or secondary plant metabolism and that the accumulation of certain metabolites could be associated with disease development and plant-pest interactions. The effects of different LED spectra on disease development was evaluated in powdery mildew (*Podosphaera xanthii*) on cucurbits, mosaic virus on tomato (*Tobamovirus*) and bacterial wilt on tomato (*Ralstonia solanacearum*) (Shuerger and Brown, 1997). The author found that red and blue LEDs were able to control diseases on various fruit. Red LED lights were found to be more effective in controlling powdery mildew in cucumber plants compared with other light sources; this effect correlated with an enhancement of the salicylic acid-dependent signaling pathway (Wang et al., 2010). Kook et al. (2013) suggested that lettuce plants grown under blue light had no symptoms

of gray mold (*Botrytis cinerea*) probably due to the development of a more compact morphology or an increase in antioxidant activity.

Vanninen et al. (2012) suggested that light colors have effects on insect behavior. The use of light to control pathogens and arthropod with less chemicals is an attractive and promising technology that has gained popularity in the agricultural sector; however, based on the studies published, the effect of light depends on cultivar and/or species.

2.12 CONCLUSIONS

Plant growth and development is controlled by light via various photoreceptors. Therefore, not to compromise, but maximise yield and nutritional quality of vegetables, growers must ensure that plants are provided with suitable light conditions. The use of LEDs in vegetables grown under controlled environment, has been recommended to regulate the light environment because LEDs are able to provide optimal integration and energy savings. Supplemental lighting can enhance greenhouse light conditions and can also reduce the level of harmful substances in certain fruit and vegetables. It is, however, advisable for growers to monitor and evaluate the provided light quality and possibly combine certain methods of modulating the light environment for plant growth, because subjecting fruit and vegetables to wrong wavelength of light can affect the accumulation of unwanted phytochemicals. Under controlled environment, the use of red and blue LEDs has an effect on growth and development of vegetables. In future, LEDs could possibly replace conventional light sources and will, therefore, be widely applied in vegetable production systems. To reduce energy consumption and achieve high nutritional value of vegetables, LEDs should be applied in combination with various light regulation strategies. The use of UV light has been shown to enhance levels of secondary metabolites compared with other types of light; however, the potentially harmful effect of UV light and the inability to regulate the emitted wavelength precisely, has so far prevented UV lights from usage in greenhouses. It is also vital to consider energy saving lights, such as LEDs, for growing plants under protected cultivation. It needs to be borne in mind that the response of plants to light treatment depends on the stage at which treatment is received by vegetables grown in a controlled environment.

The molecular mechanisms underlying the effects of light quality and quantity on changes in the levels of certain phytochemicals is also not clear. Even though the effects of light quality on quality attributes of various vegetables, particularly normal fresh tomato, have been investigated, further studies on light effects in other vegetable fruit crops like cherry tomato or on leafy vegetables are required. Therefore, future research should direct attention towards the effect of lighting on biochemical, molecular and physiological alterations in phytochemicals so as to disclose how effectively light quality manipulation can alter the development and growth of fruit and vegetables.

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CHAPTER THREE

PRE-HARVEST ALTERATIONS IN TOMATO FRUIT QUALITY FOLLOWING EXPOSURE TO RED AND BLUE LED LIGHTS

3.1 ABSTRACT

Tomato is one of the most-consumed vegetable fruit in the world; it is recognized as a good source of ascorbic acid and carotenoids, particularly β-carotene and lycopene. In preventing chronic diseases, such as cardio-vascular diseases, cancer and neuro-degenerative diseases, due to a healthy diet associated with the consumption of tomatoes, the demand of tomato has increased rapidly, in particular in supermarkets, hotels and restaurants. Poor pre-harvest practices have led to high losses and poor quality of tomatoes. Tomato pre-harvest losses due to poor management practices contribute to the high dependence on vegetable imports. Mature green fruit of the same age and injury free, with negative *a values were used for the experiment. Twelve trusses, six from each cultivar were selected to receive light treatment. Six trusses, three of each cultivar were illuminated with FLC-10W-R red LED light (RL) and another six trusses, three of each cultivar were illuminated with FLC-10W-BL blue LED light (BL). Pre-harvest red and blue lights significantly affected the measured quality attributes of the red and the yellow cultivars but affected colour and pigments more significantly. Light treatments enhanced the accumulation of lycopene on red tomatoes more that on yellow tomatoes. Red and blue lights did not significantly affect sugars and total soluble solids (TSS). Both light treatments enhanced colour change and in both red and yellow cultivars of cherry tomatoes. Light treatments not only affect colour, size and pigments, but, it was able to prevent spoilage associated with diseases on tomatoes.**

Keywords: β-carotene, cardio-vascular diseases, lycopene, red LED light (RL), blue LED light (BL)

3.2 INTRODUCTION

The sales of ready-to-use fruit, vegetables and fresh produce has grown rapidly in the past years; this is predominantly because the consumption of these foods has been demonstrated to have beneficial effects on human health (Ribeiro et al., 2012). Tomato is one of the most-consumed vegetable fruit in the world; it is recognized as a good source of ascorbic acid and carotenoids, particularly β-carotene and lycopene (Tommonaro et al., 2008).

In preventing chronic diseases, such as cardio-vascular diseases, cancer and neuro-degenerative diseases, a healthy diet is an important factor, as it assists in weight management and improves the energy balance. Several studies have demonstrated that there is an inverse correlation between the consumption of tomato and the risk of cancer (Giovannucci, 1999). Due to the health benefits associated with the consumption of tomatoes, the demand for tomato has increased rapidly, in particular in supermarkets, hotels and restaurants. However, poor pre- and post-harvest practices have led to high losses and poor quality of tomatoes (Genova et al., 2006). Tomato pre-harvest losses due to poor management practices contribute to the high dependence on vegetable imports.

Researchers have experimented with various pre-harvest treatments so as to prevent losses of fresh tomato produce associated with poor management practices. Many studies have shown that phytochemical accumulation in vegetables is significantly affected by genotype, light conditions, environmental temperature (Perez et al., 2008), irrigation and fertilization (Tiwari et al., 2013).

As these losses of fresh tomato keep on increasing, the demand of tomatoes particularly cherry tomatoes, forces tomato growers to grow tomatoes under controlled environment on a large scale so as to meet the increasing demand. The use of LEDs is gaining popularity in horticultural production, as it can assist in the production of high yield and enhance the presence of certain phytochemical compounds. Light emitting diodes have the advantage of being able to provide a small wavelength bandwidth, and, because of the small LEDs have low radiant heat, it is possible to separate the heat effect of a light source from its actual light effect. As a result, LEDs are used as light sources on pre-harvest preservation of plants. Moreover, in the food industry, food safety is of major concern during fruit production, storage, and food processing; therefore,

manufacturing and retail (supermarket and meat shops) establishments are shifting from traditional lighting, such as incandescent and fluorescent lamps, to light emitting diode (LED) to illuminate their products.

Light treatments particularly red and blue have been used to alter growth and development of plants, but there seems to be no information on colour development of fruit vegetables. There is only little information concerning illumination of cherry tomato fruit by supplemental lighting, therefore, the aim of this study was to determine the effects of pre-harvest red and blue light treatments on various cultivars of cherry tomato colour, ripening period and carotenoid synthesis.

3.3 MATERIALS AND METHODS

3.3.1 Air temperature, relative humidity, solar irradiance and photosynthetic active radiation measurements

Solar irradiance was determined with a PQS1 PAR Quantum sensor, CR 1000 (Campbell Scientific, Utah Logan, USA) (CMP3, Kipp and Zonen)). Photosynthetic active radiation (PAR) was determined using two different sensors, namely S1-111, apogee, and Li-cor, LI-190R Quantum Sensor) (Campbell Scientific, Utah Logan, USA). Apogee has some problems with wavelength colour, however post adjustments needs to be done. Air temperature and relative humidity were recorded with a Humidity and Temperature Probe HMP60 (Vaisala INTERCAP® FI-00421 Helsinki, Finland). All the measurements of air temperature, relative humidity, solar irradiance and photosynthetic active radiation were recorded every 10 s, hourly plus daily, output received and downloaded using a laptop.

3.3.2 Plant material and growing conditions

Two cultivars (‘Goldilocks’(yellow tomato) and ‘Cherry Little Wonders’ (red tomato)) a red and yellow cherry tomato (*Solanum lycopersicum*) seeds were bought from Starke Ayres (Pietermaritzburg, South Africa) and sown into two 128-cell trays filled with composted pine bark on 20 March 2017. Seedlings were kept in a temperature-controlled environment with a day/ night temperature of 24/16˚C. After three weeks, seedlings were transplanted into 3 L plastic pots filled with Gromor® (Gromor, Cato Ridge, South Africa) potting mix and the pots were placed into a glasshouse situated at the University of KwaZulu-Natal, Pietermaritzburg. The

glasshouse was equipped with a fan cooling system to control day and night temperature; it was also equipped with heat pumps to provide warm air when necessary, as plants were grown in autumn and winter. Plants were irrigated by hand with 250 ml per 3 L pot once a day, when necessary. Plants were fertilized with either one teaspoon 3:1:3 (N: P: K) or calcium magnesium nitrate plus boron once a week, until they started flowering. Training and pruning was practiced on a weekly basis to remove suckers and allow only one central leader to grow. In winter, plants were subjected to grow lights (Red LEDs within the range of 620-710 nm with special attention provided for the 660 nm wavelength, blue LEDs within the range of 400-495 nm, Ultra violet (UV) LEDs within the range of 280-400 nm, far-red LEDs within the range of 710-850 nm) from 6:00 am to 6:00 pm to extend the daylight.

3.3.3 Light treatment conditions

The day of the first flowering of the individual plants was recorded. When fruit were mature green, the *a** values [colour component of the CIELab model determining the green (negative values) or red (positive values) colour of an object] were recorded. Twelve trusses of the same age, six from each cultivar were selected to receive light treatment. Just before they were illuminated, the a* value was measured as to ensure that fruit were at the same stage of development.

Six trusses, three of each cultivar, were illuminated with FLC-10W-R red LED light (RL) and another six trusses, three of each cultivar were, illuminated with FLC-10W-BL blue LED light (BL). It was ensured that the distance from each light source to the truss was the same and it was also ensured that the light was equally distributed to every truss. Six other trusses were not subjected to any light source, and they were kept as the control. Since all plants were grown in the same glasshouse, aluminum foil was used to cover the treated trusses so that light could not interfere with the control treatment. Certain fruit were marked in each truss for analysis of external parameters or measurements (colour, size, firmness, and diseases). After every five days five fruit from each truss were harvested and utilized for destructive measurements of TSS, chlorophylls and carotenoids.

Fruit received light for eight hours per day, from 08:00 to 16:00 h for five consecutive days. Measurements were taken at day 0, after 5, 10, 15, 20, 25 days until fruit had reached the mature colour of the cultivar. Incidence of diseases and physiological disorders was recorded.

3.4 MEASUREMENTS OF QUALITY PARAMETERS

3.4.1. Size of the Fruits

At day 0, 5, 10, 15, 20 and 25 fruit size (diameter) was measured using a 150 mm vernier caliper until fruit were fully red or yellow. Five cherry tomato fruit were evaluated from each batch, treatment and replicate.

3.4.2 Colour Change

Tomato fruit colour was assessed at five (5) day intervals, both visually and with the aid of CIELAB model using a CR 400 Chromameter (Minolta Co. Ltd., Osaka, Japan). A particular part of the pericarp of each fruit was marked with permanent marker and readings of that particular portion were taken. The Chromameter was calibrated against a standard white tile prior to colour measurements. Tomato fruit skin colour was measured at three marked positions of the fruit surface. Recorded colour values were *a** [green (negative) to red (positive)] and *b** [blue (negative) to yellow (positive]. Luminous intensity (L*), which defines lightness, was also assessed. As tomato fruit ripened, the progressive colour change was described by plotting [*a**, *b**] co-ordinates on the CIELAB colour plane for each treatment on a time scale. Hue angle (H*) was also recorded. Visual colour observations corresponding to the CIELAB measurements were also made. Data were subjected to ANOVA through the use of the F-test to identify significant differences between treatments at the 5% confidence level (Genstat version17.1). Variation in colour within each treatment on day 25 was represented by standard deviation (SD).

3.4.3 Incidence of Diseases, Chilling Injury and Decay

Treated tomato fruit were evaluated visually on a 5-day interval for symptoms of decay. Samples that showed chilling injury or disease were counted; however, identification of the pathogen causing the decay was not attempted. Tomato fruit with signs of shriveling, pitting, skin blackening and with water-soaked areas, rots and mycelial growth were also recorded.

3.4.4 Analysis of Pigments

The concentration of carotenoids and chlorophylls was determined by spectrophotometry (Nagata and Yamashita, 1992) using exact absorbance readings of the entire tomato material extracted in acetone-hexane (2:3). Samples were macerated in a 100 ml acetone-hexane (2:3),

centrifuged in a table top centrifuge, the supernatant collected and its absorbance recorded at 663, 645, 505, 453 nm using a spectrophotometer (IRMECO GmbH, Germany, Model U2020). Chlorophyll a and b, as well as the total carotenoid concentrations of the sample solution were also calculated according to Nagata and Yamashita (1992). Equations used for the calculations were as below:

Chlorophyll a (mg/100 ml) = 0.999 A_{663} - 0.0989 A_{645}

Chlorophyll b (mg/100 ml) = -0.328 A_{663} + 1.77 A_{645}

Lycopene (mg/100 ml) = -0.0458 A_{663} + 0.204 A_{645} + 0.372 A_{505} - 0.0806 A_{453}

***β*- Carotene (mg/100 ml) = 0.216 A663 - 1.22 A_{645} - 0.304 A_{505} + 0.452 A_{453}**

(A_{663}, A_{645}, A_{505} and A_{453} are absorbances at 663 nm, 645 nm, 505 nm and 453 nm, respectively.)

3.4.5 Evaluation of Fruit Firmness

Firmness was manually evaluated by gently pressing fruit with the fingertips. A scale of 1 (firm), 2 (partially soft) and 3 (soft) was used to rate the firmness of the cherry tomatoes. Five cherry tomato fruit were evaluated from each batch, treatment and replicate to assess firmness.

3.4.6 Total Soluble Solids (TSS)

Total soluble solids (TSS) were determined for each sample fruit refractometrically in two replications using an Atago DR-A1 digital refractometer (Atago Co. Ld., Japan) at 20°C and TSS expressed as °Brix.

3.4.7 Statistical Analysis

All experiments were laid out in a factorial design. Results obtained were analyzed using Genstat version 17.1 and plotted with Microsoft Excel. Data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range test with differences at $P \leq 0.05$ considered significant. The unilateral paired-comparison test was used to determine significant differences for the sensory evaluation data (Roessler et al., 1978).

3.5 RESULTS

Pre-harvest treatments of 'Goldilocks' (yellow) and 'Cherry Little Wonders' (red) cherry tomatoes affected measured fruit quality parameters differently. This analysis has been conducted with the aim of determining pre-harvest treatments that enhance colour development and reduce the ripening period, while maintaining or altering cherry tomato quality attributes. Red (RL) and blue (BL) LEDs were directed onto the selected tomato trusses.

3.5.1 Colour Change in Tomatoes Illuminated Postharvest

The surface colour of tomatoes treated with red and blue lights was evaluated and compared to that of untreated cherry tomatoes (Figs 3.1 to 3.3). When a colour is expressed in the CIELAB colour spaces, L* defines lightness, a* denotes the red/green value and b* the yellow/blue value. At the beginning of the experiment (day 0), all tomato fruit were light green with similar CIELAB colour values, depending on the cultivar.

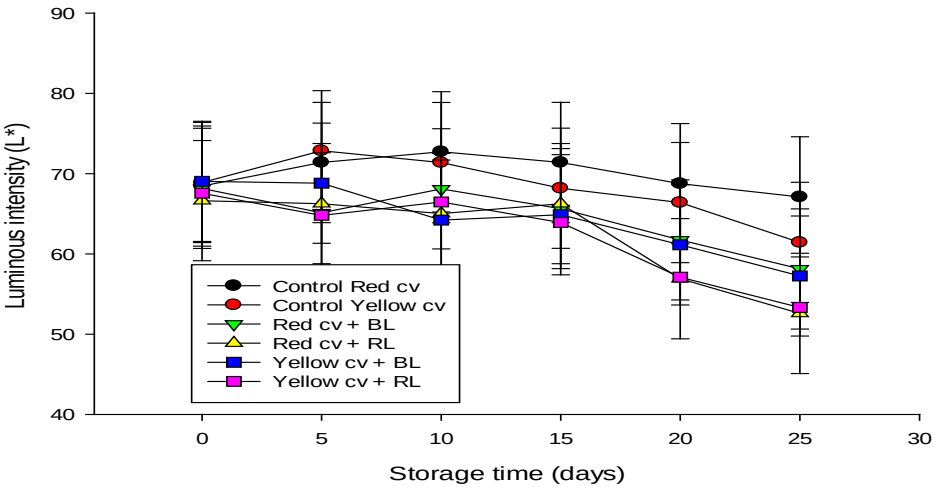

Figure 3.1: Alteration in luminous intensity (L*) of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 7.492) (BL = blue LEDs, RL = red LEDs]

Luminous intensity (L*) of tomato fruit decreased in all treatments during storage. A steady decline in L* value was recorded in non-treated fruit, while all treated tomatoes showed a rapid decline in L*. All fruit treated with red light had a tendency towards a lower L* value from day

20 to the last day of the experiment; however, no significant differences were observed between the treatments and between the cultivars of tomatoes (P > 0.05).

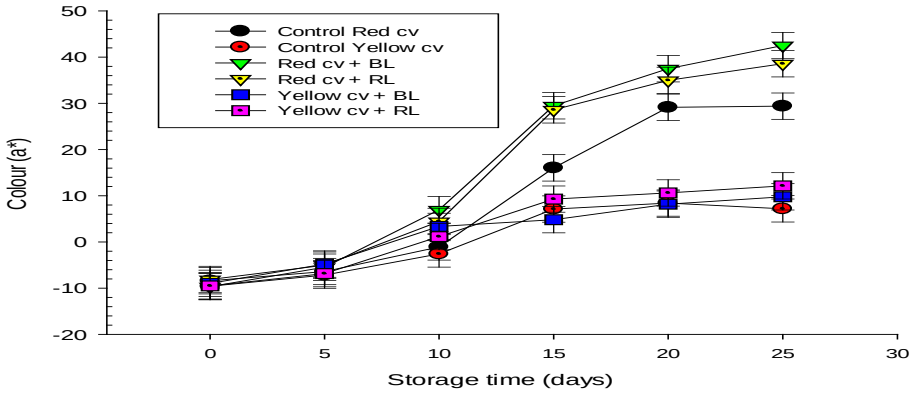

Figure 3.2: Pre-harvest alteration in red/green (a*) values of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 2.859]

All fruit were green initially. With a* value of the red and the yellow cv increased as expected. The a* values of untreated and treated tomatoes increased steadily during the initial 5 days, followed by a rapid increase from 5 to 20 days (Fig. 3.2). The red cv had a higher a* value from day 15 to 25 days, while the a* value of the yellow cultivar did not change significantly from day 15 onwards. Overall, a significant difference was overall observed between the treatments and cultivars (LSD ($P_{0.05}$) = 2.859).

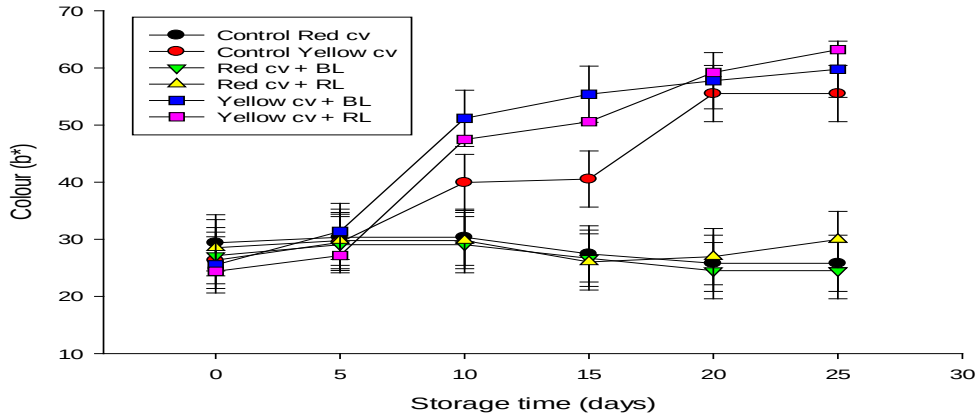

Figure 3.3: Alteration in yellow/blue value (b*) of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 4.92]

The illuminated fruit and the control of the red cv had a lower b* value from day 10 onwards. The treated fruit of the yellow cultivar had the highest b* values from day 10 onwards, but from day 20 onwards, there was no significant difference in b* value between the treated and non-treated red tomatoes and the treated and non-treated yellow tomatoes; however, treatments and control of the red cv had lower b* values from day 10 onwards (Fig. 3.3).

3.5.2 Change in fruit diameter

Initially all fruit were of the same size depending on the cultivar, with fruit of the red cultivars being significantly ($P < 0.05$) larger than those of the red cultivar. Only a tendency towards an increase in size was observed in all treatments from day 0 to day 25 (Fig. 3.4). As from day 15 all treated fruit had a bigger size compared to the control but there was no significant difference between the treated and non-treated fruit per cultivar [LSD ($P_{0.05}$) = 2.152].

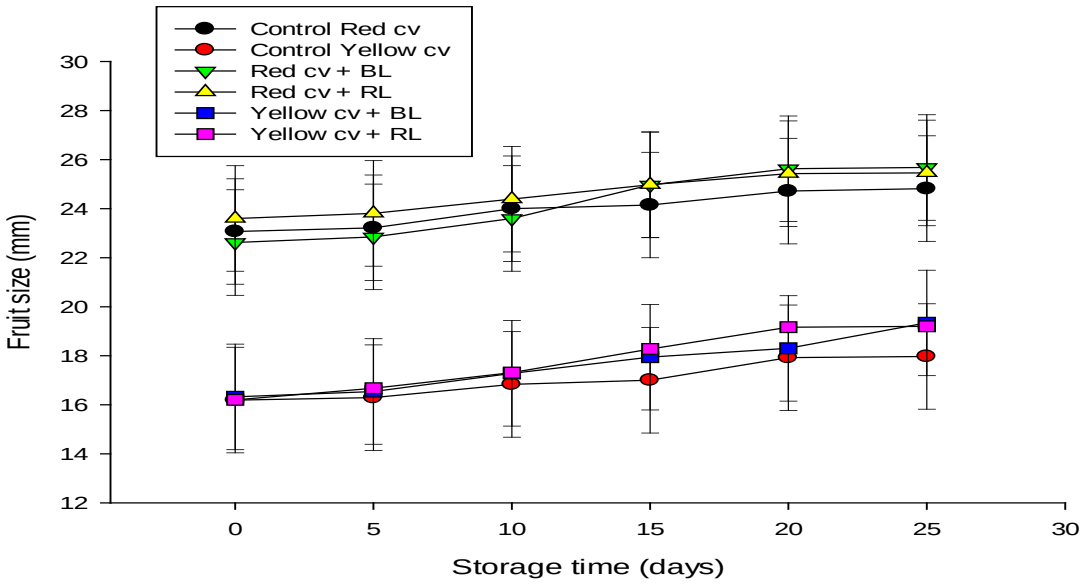

Figure 3.4: Alteration in fruit diameter (mm) of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 2.152]

3.5.3 Analysis of Pigments

Chlorophyll a concentrations differed significantly ($P < 0.01$) between control and treated fruit, with the control of the red cultivar maintaining the highest Chl a values. From day 0 to day 15, a sharp decline in Chl a was observed in all treated fruit, but fruit treated with blue or red light showed a faster decline in chlorophyll a than the control. The change in Chl a was not significant during the last five days in all treatments (Fig. 3.5). A significant difference was observed within the treatments [LSD $P_{0.05}$) = 0.003].

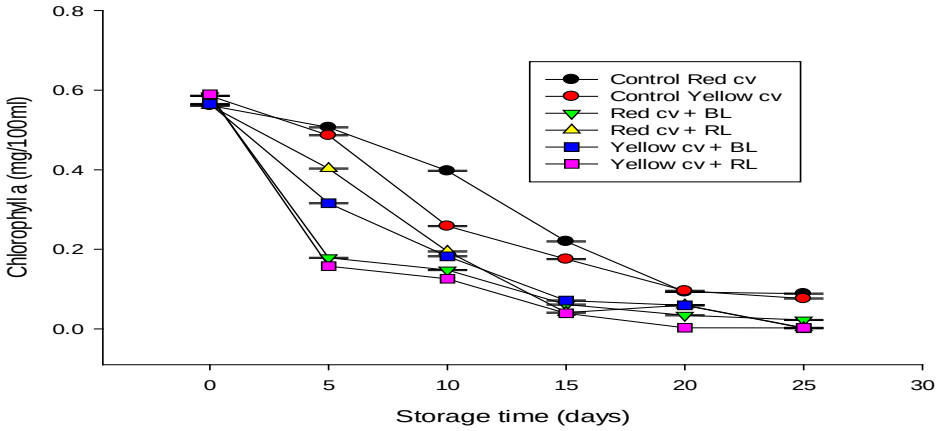

Figure 3.5: Changes in chlorophyll a concentration (mg/ 100 ml extract) of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 0.003]

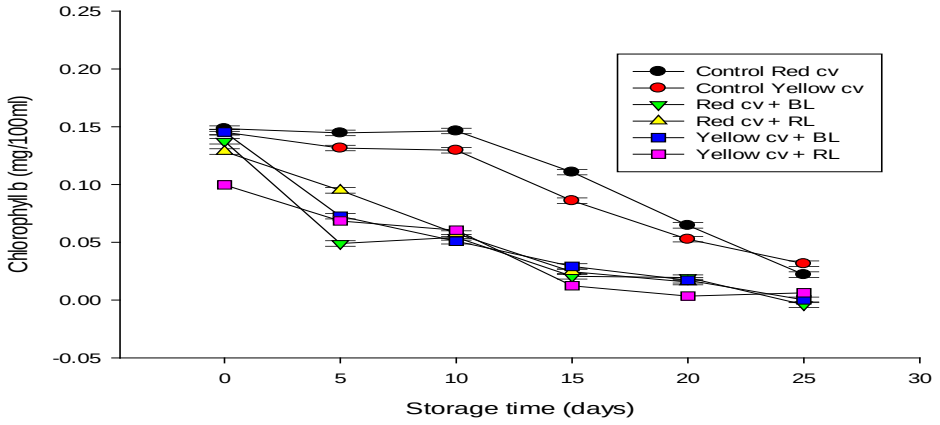

Figure 3.6: Changes in chlorophyll b concentration (mg/ 100 ml) of red and yellow cv of cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 0.004]

The fruit chlorophyll b concentrations were generally lower than the chlorophyll a concentrations. Unlike the chlorophyll a, chlorophyll b concentrations decreased more rapidly than the control in all treatments. All treatments that included blue light displayed a sharp decrease in chlorophyll b over the first five days. No significant difference was observed between the treated fruit [LSD ($P_{0.05}$) = 0.004].

There was no significant difference in lycopene concentration between the treated and non-treated fruit in the first 5 days [LSD (P0.05) = 0.001]. After five days the control and the red light-treated fruit of the red cv displayed a rapid increase in lycopene concentration. Five days later, the blue light treated fruit showed a similar increase in lycopene concentration. A significant difference was observed between the treatments ($P < 0.05$). The blue light outperformed the red light resulting in the highest fruit lycopene concentrations. Red light treatment resulted in red tomatoes with the highest lycopene concentration. The yellow cultivar, however, had a higher lycopene concentration when treated with blue light (Fig. 3.7).

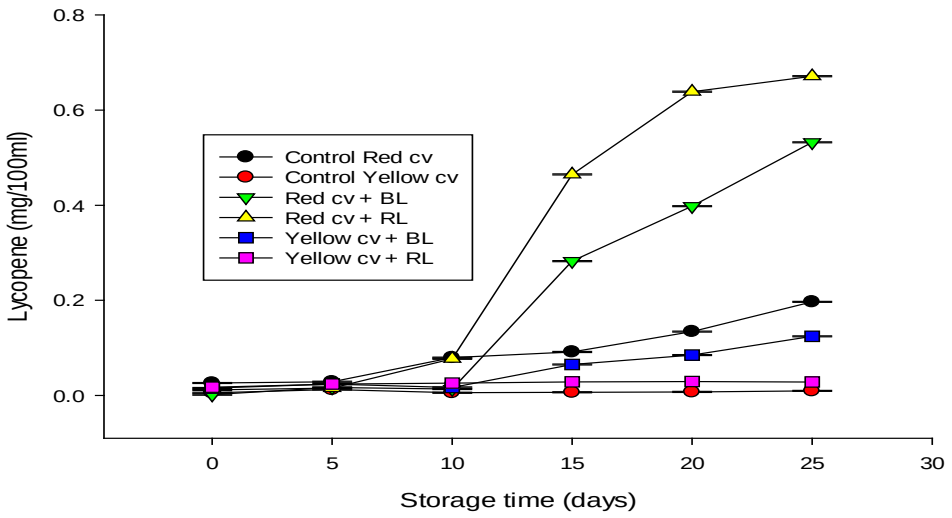

Figure 3.7: Changes in lycopene concentration (mg/100 ml) of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 0.001]

The β-carotene concentration of tomatoes, untreated and treated with red or blue light during 25 days of the experiment differed significantly from day 5 (Fig. 3.8). For the first five days all fruit displayed an increase in β-carotene, thereafter the concentration of β-carotene of all yellow fruit decreased to day 10. Control fruit of both cultivars showed only minor alteration in β-carotene after day 10, while all treatments displayed an increase from day 5 to day 20 and 25.

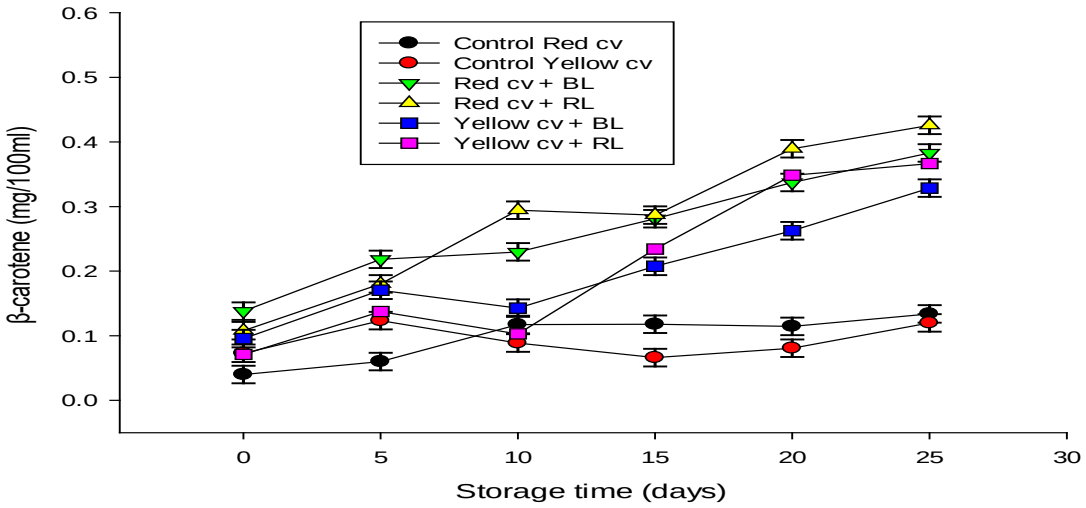

Figure 3.8: Changes in β-carotene concentration (mg/100 ml) of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 0.014]

3.5.4 Total soluble solids

The °Brix of all tomatoes did not differ significantly for the first 10 days ($P > 0.05$). The yellow cv had higher TSS during the first 10 days of storage, with both light treatments resulting in a higher TSS than the control. For the red cultivar, red light showed a similar effect, but delayed by 5 to 10 days. After 15 days of storage, TSS was higher in all treated fruit than in the controls and remained higher than the control over the last 10 days of the observation period. A significant difference was observed between the treated and non-treated fruits from day 15 to day 25 (Fig. 3.9).

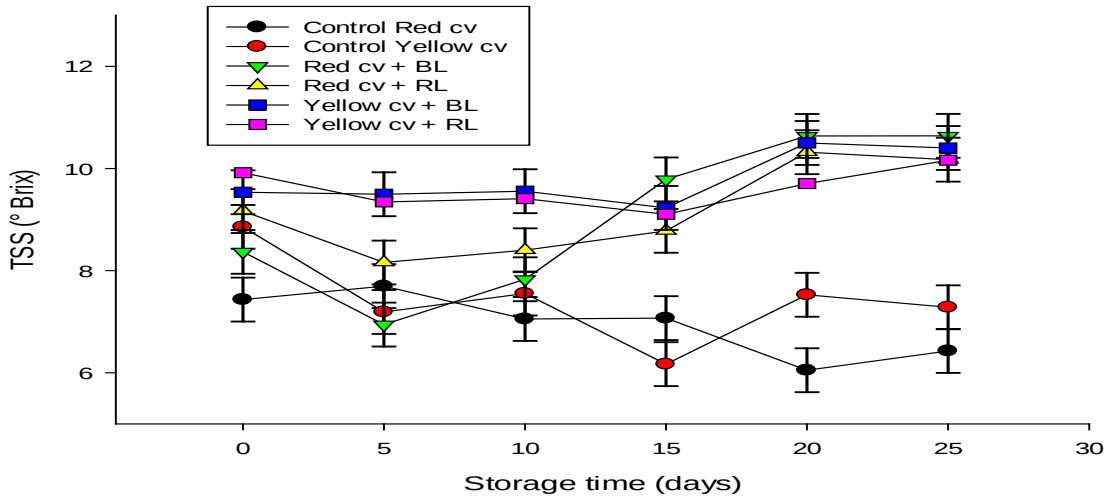

Figure 3.9: Changes in TSS (° Brix) of juice extracted from content of red and yellow cherry tomato fruit following various pre-harvest light treatments [LSD ($P_{0.05}$) = 0.430]

3.5.5 Firmness

Firmness of cherry tomatoes decreased after pre-harvest treatments, and the cherry tomato tissue became softer. There was, however, no significant difference among treatments (P > 0.05). For the first 5 days all fruit were firm, thereafter firmness of treated fruit decreased rapidly from day 5 onwards. On day 15, most treated fruit were partially soft, while the control lost firmness steadily (Fig. 3.10).

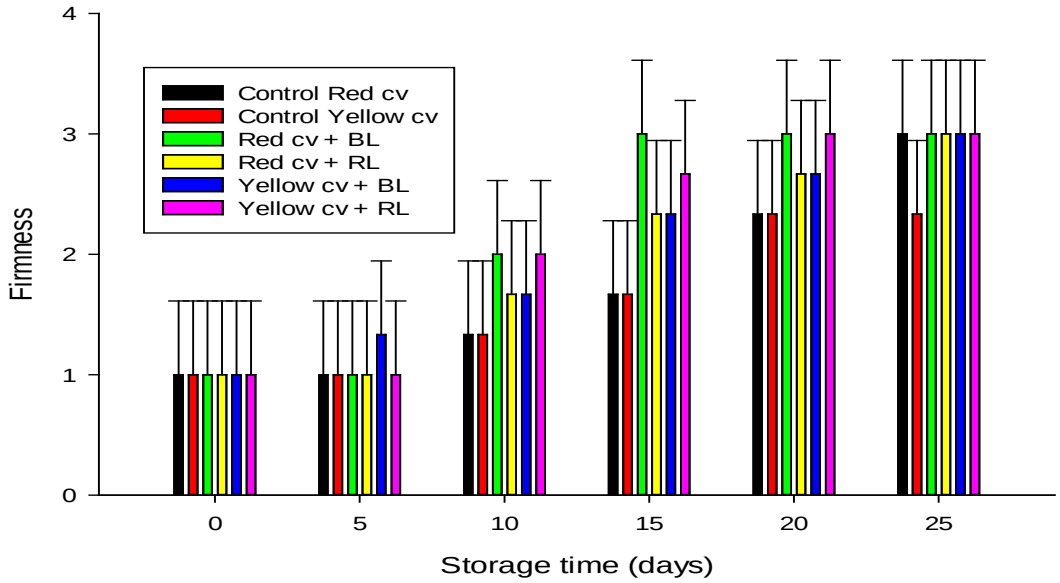

Figure 3.10: Firmness of yellow and red cherry tomatoes following various pre-harvest light treatments [LSD ($P_{0.05}$) = 0.611]

3.6 DISCUSSION

Consumption of fresh fruit and vegetables is of vital importance for the maintenance and health of humans, as these products contain a high concentration of beneficial phytochemicals like vitamins, antioxidants and sugars (Chang et al., 2013). Various authors have demonstrated that light and environmental temperature can significantly affect the concentration of phytochemicals and colour of fruit and vegetables (Mou, 2009; Liu et al., 2009; Tiwari et al., 2013). In this study, pre-harvest red and blue lights significantly affected the measured quality attributes of two cultivars (‘Cherry Little Wonders’ and ‘Goldilocks’), a red and yellow cherry tomatoes respectively.

Fruit quality and mass determine the economic yield of tomatoes. Fresh mass of individual fruit is an important quality aspect with each size grade having a different market price (Marcelis, 1998). Light treatments did not have a significant effect on fruit size ($P > 0.05$); initially all treated fruit were of the same size, only differing according to cultivar. The size of all light-

treated fruit was bigger than that of untreated fruit from day 15 to day 25. However, there was no statistical significant difference between treated and non-treated fruits ($P > 0.05$). Lu (2012) reported that blue light exposure improves the first two truss yield of tomatoes, where supplemental light was applied for 28 days during the rapid fruit development stage. Other authors also demonstrated that blue + red (1:1) and blue LEDs can improve fresh mass of young lettuce (Trouwborst et al., 2010; Fan et al., 2013; Samuoliene et al., 2013; Chen et al., 2014) compared with red lights. Light emitting diodes (LEDs) have several unique properties, producing high light intensity, but excluding the heat that is given off by incandescent lights. This additional heat can positively affect fruit size, if the temperature surrounding the tomato plants is suboptimal. It is positive that the LED treatment did not decrease size, which could have pointed to stressful conditions subjected to cherry tomato fruit.

The appearance of tomato fruit is affected by the alterations in pigments during ripening (Salunkhe et al., 1974). Skin colour and texture are one of the most important and complex attributes of tomato fruit quality (Liu et al., 2011). The characteristic that determines the degree of consumer's acceptance is firstly colour, while the following quality parameter consumers judge tomatoes on is taste and firmness; this parameter ultimately makes the consumer decides to buy fresh tomato (Pinheiro et al., 2013). One of the most crucial factors for buying tomatoes from a certain retailer, is flavour (León-Sánchez et al., 2009), but in the last decades, commercial tomatoes have been criticized for lacking desirable flavour (Krumbein et al., 2004).

The various sequences of colour change, observed in cherry tomato fruit treated with different treatments (Figs. 3.1 to 3.3), could be the result of the varying rates of chlorophyll degradation and carotenoid formation. Tomato fruit treated with LED lights seemingly showed a faster decline in chlorophyll a, and particular chlorophyll b (Fig. 3.5 and 3.6) than the control, resulting in faster colour change. This explains the conversion from green to red/yellow colour of cherry tomatoes. Additionally, lycopene and β-carotene concentrations increased following red and blue light exposure, particularly in the red cultivar. A study by Li and Kubota (2009) demonstrated that stress increases the concentration of lycopene and β-carotene.

Not only was the red-green colour parameter of the tomatoes affected by the light treatment, but also fruit lightness, L*. Following treatment, L* steadily decreased in treated and untreated tomato fruit for the first 10 days. Thereafter, a rapid decrease in L* was observed. During

ripening, the colour of tomatoes changes and these changes are the result of two simultaneous processes, degradation of chlorophyll and synthesis of carotenoids (particularly lycopene and β-carotene (Radzevicius et al., 2009). Fruit were treated at the mature green stage (a* value ranging from -9 to -10, and b* value from 25 to 30). A study by Li et al. (2009) revealed that the concentration of carotenoids increased by 12%, phenolics increased by 6% in baby lettuce exposed to supplemental blue light. Dhakal and Baek (2014) also reported that colour development of tomatoes can be enhanced by red light (at 650-660 nm). The authors found that red light enhanced color of red tomatoes and increased the concentration of lycopene during 21 days of storage in darkness. A study by Maharaj et al. (1999) demonstrated that tomatoes irradiated with UV-C (3.7 kJ/m^2) significantly delayed senescence and colour development. Yellow cultivar had a lower a* value and higher value of b*(green to yellow) from day 10 to day 25. A steady decrease in colour b* was observed in the red cv of tomato while a sharp increase was observed in the yellow cv, while fruits that were illuminated with red light had a higher b* value on both cultivars. The higher b-value could come from xanthophylls which are yellow pigments or β-carotene. Coloring in yellow cherry tomatoes is enhanced by a recessive mutant gene. Yellow cherry tomatoes have no detectable anthocyanins, which is the compound that is responsible for red pigmentation and they have low concentration of chlorophylls unlike red cherry tomatoes. The concentration of flavonoids in the skin of yellow cherry tomatoes, carotene or yellow carotenoids is very high and it results in yellow coloration (Tijskens and Evelo, 1994).

During ripening, most fleshy fruit lose their green colour and accumulate various pigments that provide a distinctive colour to the ripe fruit. Seed dispersing animals are attracted by these pigments (Klee and Giovannoni, 2011; Seymour et al., 2013; Zhong et al., 2013). Out of the three major groups of plant pigments, namely, carotenoids, betalains and anthocyanins; carotenoids are very important for plant life as photoprotectants of chlorophyll (Fraser and Bramley, 2004; Ruiz-Sola and Rodriguez-Concepcion, 2012). Enhancing the production of carotenoids in tomato fruit contributes to the visual change in colour during the ripening process. As a result, the colour of mature green tomato changes from green to red or orange when ripe, due to the accumulation of the yellow xanthophylls, the orange carotenoid β-carotene, and the red carotenoid lycopene in the pericarp of the fruit. This synthesis must, however, be accompanied by the breakdown of chlorophylls (Tomato Genome Consortium, 2012; Fantini et

al., 2013; Seymour et al., 2013). Carotenoids also increase the nutritional quality of the fruit, as they serve as the precursors for the production of retinoids and they also provide some health benefits as antioxidants and anti-cancer agents (Fraser and Bramley, 2004; Ruiz-Sola and Rodriguez-Concepcion, 2012).

A sharp decrease in chlorophyll concentration and a corresponding increase in carotenoid synthesis during the fruit ripening process was observed (Figs. 3.5 to 3.8). This change in pigment profile is aligned with plastid conversion from chloroplasts to chromoplasts. Chlorophyll a, b and carotenoid concentrations in tomato differed significantly ($P < 0.01$) between treatments, with the control maintaining the highest Chl a and Chl b values until day 25. There was a statistical significant difference between untreated and treated fruit in terms of changes in Chl a and b ($P < 0.05$) (Figs. 3.5 and 3.6). In the first 10 days, the red cv treated with BL and the yellow cv treated with RL showed a rapid decrease in Chl a. Chlorophylls a and b are the major green pigments of tomato fruit and they take part in the photosynthetic process during growth and maturation. A significant difference was observed between the treated fruit [LSD (P0.05) = 0.003], after 10 days the rate of change of Chl a was the same, where no significant difference was observed ($P < 0.05$). A steady decrease in Chl b concentration of the treated fruit was observed from day 5 to day 25 and there was no significant difference of the treatment effect on fruit ($P < 0.05$). Previous studies have demonstrated that environmental factors, such as light, have a significant effect on fruit ripening (Azari et al., 2010). Light treatments are able to facilitate chlorophyll a and b breakdown, however, while Chl a is broken down more easily Chl b needs to be converted to a, before it is broken down (Hörtensteiner and Kräutler., 2011; Seymour et al., 2013). According to Muller et al. (2001), reactions for the formation of pigments depend on the metabolic energy provided by ATP. It seems probable that fruit stored in the light made use of additional energy provided by the LED light to increase the rate of pigment synthesis, enabling faster appearance of pigments in illuminated tomatoes.

The accumulation of carotenoids in tomato was accompanied by a sharp decline in chlorophylls. Chlorophyll degradation and lycopene accumulation are the most important processes during tomato fruit ripening and senescence. The accumulation of lycopene commenced in treated tomatoes 10 days after treatment, but for the first 10 days there was no statistical difference between the treated and non-treated fruit ($P < 0.05$). A dramatic increase in lycopene

concentration was observed in the red tomato cv after 10 days, where red light was more influential in enhancing the lycopene concentration compared with blue light.

The lycopene concentration of yellow tomatoes was lower that of red tomatoes (Fig. 3.7). This is in line with Walter and Strack (2011) who reviewed lycopene metabolism and described the yellow xanthophylls to be derived from the lycopene. While the same authors also elaborated on the importance of light for lycopene biosynthesis, only few studies have reported on the effect of red and blue light on cherry tomato irradiated while still attached on the plant and allowed to ripen on the tree. A study by Alba et al. (2000) demonstrated that fruit-localized phytochromes which are activated by red light, regulate the biosynthesis of lycopene in tomato tissues as a result of chlorophyll breakdown. Liu et al. (2009) demonstrated that accelerating chlorophyll biodegradation can be achieved by illuminating tomato fruit with red light. The biosynthesis of carotenoids during ripening, on the other hand, can be enhanced by blue light, as red light is required to activate phytochrome to be able to activate ripening. The absorption maxima of carotenoids lie in the blue light region; hence, the biosynthesis of carotenoids was accelerated (Appendix A). The concentration of β-carotene was higher in fruit of the yellow cv treated with both, red and blue lights compared with the red cultivar. The potential implication of administering such light treatments to tomatoes could result in fruit with higher β-carotene concentrations, assisting in the provision of provitamin A, important to increase the daily intake of vitamin A (West et al., 1999). Red light (625-700 nm) was able to increase the concentration of carotenoids in green baby leaf lettuce (Zukauskas et al., 2011) and blue light was able to do the same on standard lettuce (Stutte et al., 2009). In fruit, the distribution of carotenoids is not regular, in the sense that the concentration of lycopene is higher in the tomato pericarp than in the locules, with a higher concentration of β-carotene in the locules than the pericarp (Davies et al., 1991).

As the degradation of chlorophyll concentration and accumulation of lycopene and β-carotene occurs in tomato fruit, the fruit tends to lose firmness and becomes soft. A decrease in the concentration of the chlorophylls in the fruit causes the fruit to become softer. This feature is correlated with increasing maturity and, therefore, traditionally used as a criterion for visual assessment of fruit maturity (Manning, 1993).

The firmness of cherry tomatoes decreases during storage because the tissue becomes soft due to metabolic changes induced by enzymatic reactions and respiration breaking down the cell walls and plasmamembranes. Softening of tomato fruit is of great economic importance for both producers and consumers, as softer fruit have a lower market value because of lower storability. In this study, the firmness of treated and non-treated fruit was similar (Fig. 3.10) and the same in all fruit for the first five days postharvest, except in the yellow cv treated with BL. This treatment lost firmness most rapidly. Five days after picking, treated tomato fruits gradually decreased in firmness compared with untreated fruit that did not receive light. On day 20 fruit that received light treatment were already soft and few were partially soft. On day 25 all fruits were soft and both blue and red lights had no significant effect on hardness of tomatoes. The treatment positively affected colour without having a negative effect on firmness and that, therefore, such a treatment should be tested on a semi-commercial basis.

There was no statistical difference between treated and untreated fruits ($P < 0.05$) for firmness. Other studies have demonstrated that red and blue light have varying effect on firmness of the fruit and that red light increases the carotenoid content (Lee et al., 1997). A study by Alba et al. (2000) demonstrated that firmness loss in the tomato pericarp is not influenced by the exposure to red or red/far-red light; however, it is believed that the use of blue light treatment stimulate the biochemical processes of cherry tomato as a result the fruit become softer. The blue light has higher energy and is more destructive, breaking down cell components (membranes, cell walls) thereby accelerating softening.

Flavour plays an important role in tomato fruit. Sugars and acids contribute to tomato flavour, while total soluble solids are predominantly sugars. In general, the flavour of a fruit becomes pronounced when its sugar content peaks (Salunkhe et al., 1974). In this study there was a significant ($P< 0.05$) difference in TSS between treated and un-treated tomato fruit (Figure 3.9). Initially, the TSS level of the red fruit was lower than the yellow tomatoes. As storage time progressed, alterations in TSS did not show a clear trend. The °Brix value of treated fruit was similar from day 20 to day 25, while the °Brix values of untreated fruit was very lower (Fig. 3.9). A study by Liu et al. (2009) is in contrast with the present study. The authors demonstrated that red light exposure has a minimal effect on TSS of standard tomatoes.

Light does not only affect pigments in plants, it can also prevent the occurrence of diseases and disorders. Many studies have recorded LEDs to have a positive effect on plant photosynthetic characteristics, physiological metabolism, and fruit quality (Chen et al., 2009). In addition, LEDs of various wavelengths can improve fruit or plant resistance to stress and regulate fruit defence mechanism (Ballare, 2014). Kim et al. (2013) demonstrated that *Botrytis cinerea* development in tomatoes can be significantly supressed by the use of blue LEDs. Other authors have also discovered that red light can improve plant resistance and induce resistance to many types of diseases (Islam et al., 1999). A study by Ridker and Kook et al. (2013) demonstrated that the control efficacy of diseases (*B. cinerea*) in lettuce is associated with an increase in antioxidant content as well as the development of compact morphology by blue-light treatment. In the present study blue and red lights were able to control the diseases as there were no symptoms of diseases, physiological disorders and chilling injuries compared to the control fruit. Another study by Shuerger and Brown (1997) evaluated the effects of different LED spectra on disease development in powdery mildew (*Podosphaera xanthii*) on cucurbits, mosaic virus on tomato (*Tobamovirus*) and bacterial wilt on tomato (*Ralstonia solanacearum*). The authors found that red and blue LEDs were able to control diseases on various fruit. This proves that with more antioxidants, particularly carotenoids, fruit are able to fight pathogens and fight diseases.

3.7 CONCLUSIONS

From the present study, it can be concluded that exposure to red and blue light enhances the accumulation of lycopene in red tomatoes more than in yellow tomatoes with minimal effects on firmness. The β-carotene concentration was enhanced by both, red and blue light, but more so in yellow than in red cherry tomatoes. This indicates that light (red and blue wavelengths) can be used as a driver of carotenoid synthesis and accumulation in tomatoes. Light treatment was able to moderately increase soluble sugars in tomatoes prior to commercial maturity. It can be concluded that ripening, colour and carotenoids can be enhanced in red and yellow cultivars of cherry tomatoes. Such treatment does not negatively affect internal quality parameters. As the choice of wavelength of light treatment should bear the final colour of the tomato in mind, tomato growers must make sure to use the correct light at the correct maturity stage. The use of light

emitting diodes is promising for greenhouse horticulture, but further knowledge must be acquired on the effects of different wavelengths of LEDs on various vegetables prior to usage of the technology on a larger commercial scale. It would also be interesting to study the effect of these treatments of LEDs on ascorbic acid, the most potent phytochemical in plants.

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CHAPTER FOUR

POST-HARVEST ALTERATIONS IN TOMATO FRUIT QUALITY AT DIFFERENT STAGES FOLLOWING EXPOSURE TO RED AND BLUE LED LIGHTS

4.1 ABSTRACT

Tomato is a climacteric fruit, as it continues to ripen, even if detached from the mother plant. During ripening of tomatoes, carotenoid synthesis is accompanied by the degradation of the green pigment chlorophyll. Lycopene and β-carotene are the major carotenoids in ripe tomato and represent the primary components of ripe fruit pigmentation conferring deep red or orange colour, respectively, to the fruit. The effects of red and blue light treatment on colour, ripening, carotenoids and quality of two cultivars treated at different fruit ripening stages was valuated in this study. Light treatments were able to enhance colour development, carotenoids and total soluble solids (TSS) more on cherry tomato fruits treated at mature green stage. Yellow cultivar on both stages (mature green and turning) had a lower lycopene content compared to red cultivar. There was no significant difference ($P < 0.05$) in change in mass of fruit that received red and blue lights and non-treated fruits. Treating tomato fruits at mature green stage with post-harvest light could enhance colour development and more pigments and carotenoids with less effect on mass loss compared to treating the fruits at the turning stage.

Keywords: β-carotene, cherry tomato, lycopene, mature green, turning stage

4.2 INTRODUCTION

Tomato is one of the most important fruit due to its colourful appearance as well as its health benefits. Tomato is a climacteric fruit, as it continues to ripen, even if detached from the mother plant. During ripening of tomatoes, carotenoid synthesis is accompanied by the degradation of the green pigment chlorophyll. Lycopene and β-carotene are the major carotenoids in ripe tomato and represent the primary components of ripe fruit pigmentation conferring deep red or orange colour, respectively, to the fruit. The most important quality parameters in tomatoes are colour and texture; they relate directly to the fruit marketing value (Tijskens and Evelo, 1994).

Carotenoids are not only important due to the colour they impart, but also due to certain health benefits. Various epidemiological studies demonstrated that tomato carotenoids play a significant role in reducing the incidence of degenerative diseases and the prevention of diseases, such as cancer, cataracts, and heart disease (Agarwal and Rao, 2000). By developing postharvest technologies that are able to intervene with ripening and certain breeding programmes, tomato researchers have been trying to enhance the levels of carotenoids in the fruit (Alba et al., 2000; Rosati et al., 2000; Liu et al., 2003). Light treatment is one of these ripening intervention technologies that can be applied pre- and postharvest. Greenhouse managers adopt techniques to improve crop production, among these are light emitting diodes (LEDs). These LEDs have several unique properties, producing high light intensity without giving off large amounts of heat, unlike incandescent lights. Further, LEDs have a narrow bandwidth; therefore, offering the possibility to control spectral composition very specifically (Bourget, 2008).

Earlier studies have indicated that phytochrome induces carotenoid biosynthesis in tomato by perceiving red light and activating biochemical changes via the bioactive phytochrome far-red (Pfr) (Thomas and Jen, 1975). A study by Alba et al. (2000) demonstrated that subjecting tomato to red LED treatments (six 40 W Gro-lux lamps) can increase lycopene accumulation, while far-red light can reverse the lycopene accumulation induced by red light. A study by Lee et al. (1997) demonstrated that the carotenoid content of red tomatoes can be increased by red light treatment, with varying effects on tomato firmness. Light emitting diodes (LEDs) do not only enhance the accumulation of carotenoids and degradation of chlorophylls, but also prevent the occurrence of diseases in fruit. The above-mentioned authors described that certain lighting systems could

significantly reduce the occurrence of fungi and other pathogens as well as insects on certain fruit and vegetables. Not all wavelength are, however, able to reduce the ability of these organisms to multiply, or of insects to feed on the host species and reproduce (Massa et al., 2008). Red LED lights were found to be more effective in controlling powdery mildew in cucumber plants compared with other light sources (Wang et al., 2010).

There is limited information on the effects of different wavelengths on various tomato cultivars. Most authors focus on standard tomatoes. The aim of this study was, therefore, to investigate the effects of post-harvest red and blue LED light treatments on two cultivars of cherry tomatoes, red ('Cherry Little Wonders') and yellow ('Goldilocks') which received light at different stages of development, as postharvest.

4.3 MATERIALS AND METHODS

4.3.1 Air temperature, relative humidity, solar irradiance and photosynthetic active radiation measurements

As described in section 3.3.1.

4.3.2 Plant material and growing conditions

As described in section 3.3.2.

4.3.3 Light treatments

Tomato fruit of both cultivars ('Cherry Little Wonders' and 'Goldilocks') were harvested at different stages of development, namely at the mature green stage and at the turning stage. Tomatoes were deemed green mature at a* values ranging from -9 to -12 for both cultivars (green (negative) to red (positive)). A further batch of tomatoes was removed from the plant and received light treatment, when fruit were mature pink (a* value between 5 to 10 for the red cv and 20-30 for the yellow cv). It was ensured that fruit used in the experiment were of the same size and shape and injury-free. Harvested fruit were sorted and randomly grouped into batches of five fruit per cultivar and subjected to various treatments. Harvested tomato fruit were exposed to either red LED (RL) or blue LED (BL) lights for the same duration, and packaged into macro perforated plastic bags.

The following treatments were administered before storage: red light (RL), blue light (BL), and all fruits were packed in modified atmospheric packaging. Each treatment consisted of 90 fruit. One batch of each cv was illuminated with FLC-10W-R RL for 48 h, a further batch of each cv was illuminated with FLC-10W-BL BL for the same duration. It was ensured that the distance from the light source to the fruit was equal for every illumination. Fruit in each of these treatments were packaged into perforated plastic bags and the bags were sealed. A control treatment was also packaged before storage. Fruit were stored for 21 days at 23°C and from each tomato batch, treatment and replicate, five fruit were sampled for analysis. Fruit mass, colour, firmness, total soluble solids (TSS) and pigment concentrations were determined as quality attributes at 5-day intervals over a 21 day experimental period.

4.4 MEASUREMENTS OF QUALITY PARAMETERS

4.4.1 Change in mass

Tomato fruit batches of 30 fruits in each replication were weighed at the commencement of the experiment and during storage and mass loss relative to the initial value was calculated and expressed in percentage using the formula:

$\frac{(\mathrm{mi}-\mathrm{mf})/}{\mathrm{mi}} \times 100$, where m_i was the initial weight and m_f was the sample weight

The mass of the fruit was recorded in five day intervals. Five fruit were weighed and the average mass was recorded for each batch, treatment and replicate.

4.4.2 Colour assessment

Colour was assessed on the marked parts of fruit from each batch, treatment and replicate as described in section 3.4.2.

All the following parameters were assessed as described in sections 3.4.3- 3.4.7:

4.4.3 Incidence of diseases, chilling injury and decay

4.4.4 Pigments

4.4.5 Firmness

4.4.6 Total soluble solids (TSS)

4.4.7 Statistical analysis

4.5 RESULTS

Postharvest blue and red light treatments of cherry tomatoes at the mature green and turning stages affected measured fruit quality parameters.

4.5.1 Change in mass of fruit

Mass loss was observed in all the treatments, but the rate of loss varied between treatments [LSD ($P_{0.05}$) = 0.0005] (Fig. 4.1). Mass loss was faster in all treatments for the first 10 days then thereafter. At the turning stage (Fig. 4.2) a similar, steady increase in mass loss was observed.

There was no significant difference between the treatments in both mature green and turning fruit ($P > 0.05$). Light treatments did not have a negative effect on fruit mass on both stages.

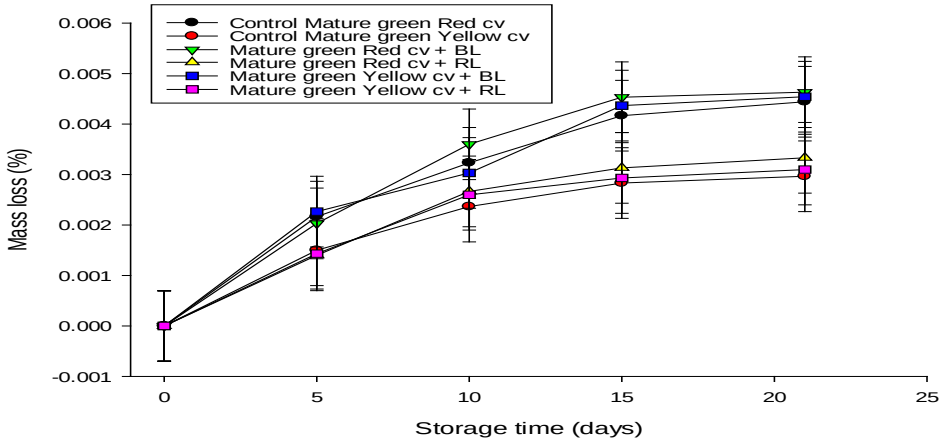

Figure 4.1: Change in fruit mass (%) of red and yellow cherry tomatoes treated postharvest at the mature green stage with various LED lights and stored at room temperature over a 21-day storage period [LSD ($P_{0.05}$) = 0.0005]

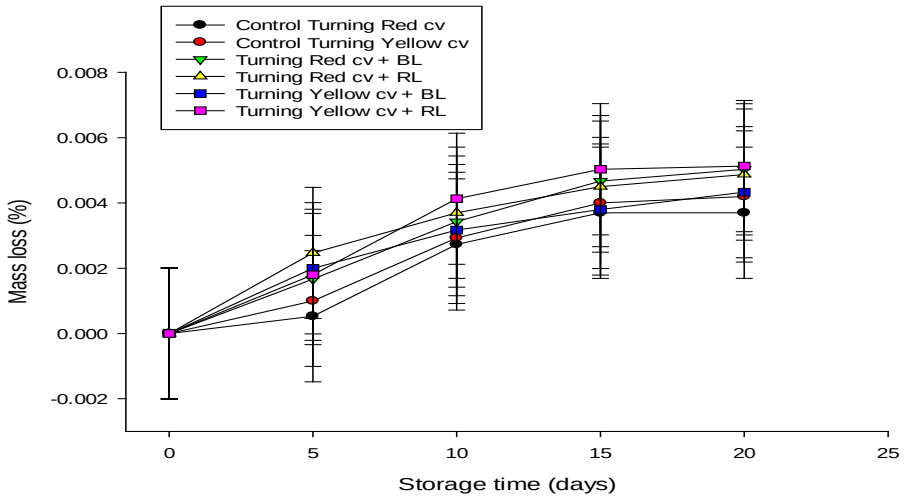

Figure 4.2: Change in fruit mass (%) of red and yellow cherry tomatoes treated postharvest at the turning stage with various LED lights and stored at room temperature over a 21-day storage period [LSD ($P_{0.05}$) = 0.0005]

4.5.2 Colour change in tomatoes illuminated postharvest

During postharvest storage and ripening, the colour of tomato fruit changed daily, as represented by the distance between successive a*, b*, and L coordinates plotted on the CIELAB colour plane or in individual 2-dimensional graphs. At the beginning of the experiment (day 0), all tomato fruit were either mature (Figs. 4.3 to 4.5) or turning (Fig. 4.6 to 4.8) and had similar CIELAB colour values, depending on the maturity stage.

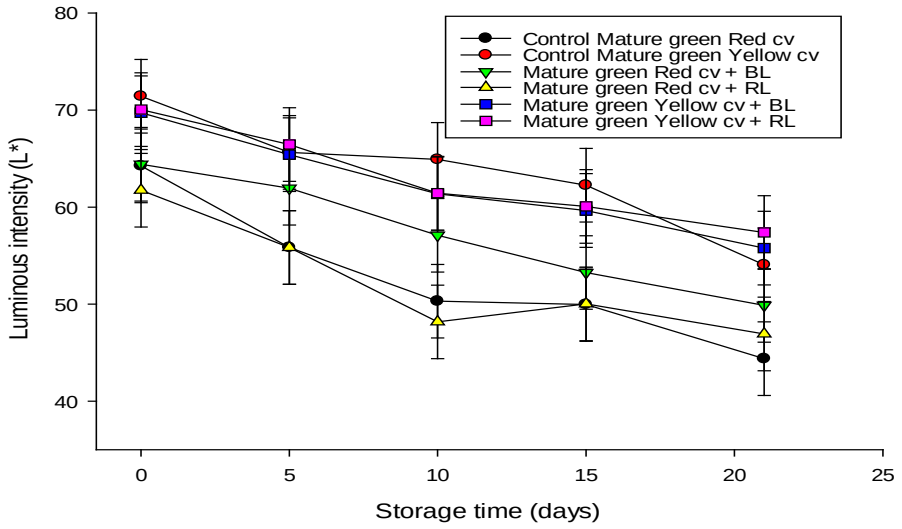

Figure 4.3: Luminous intensity (L*) of red and yellow cherry tomato fruit treated postharvest at the mature green stage with various LED lights [LSD ($P_{0.05}$) = 3.793]

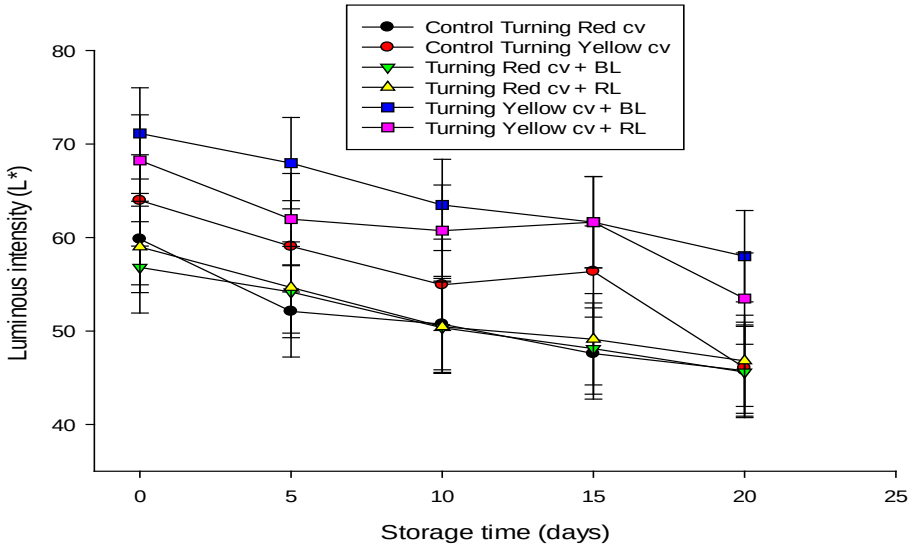

Figure 4.4: Luminous intensity (L*) of red and yellow cherry tomatoes at the turning stage treated postharvest with various LED lights [LSD ($P_{0.05}$) = 4.883]

Luminous intensity (L*) of tomato fruit decreased in all treatments during storage (Figs 4.3 and 4.4). A rapid decline in L* value was recorded in the control treatments. Significant difference were observed between the treatments ($P < 0.05$).

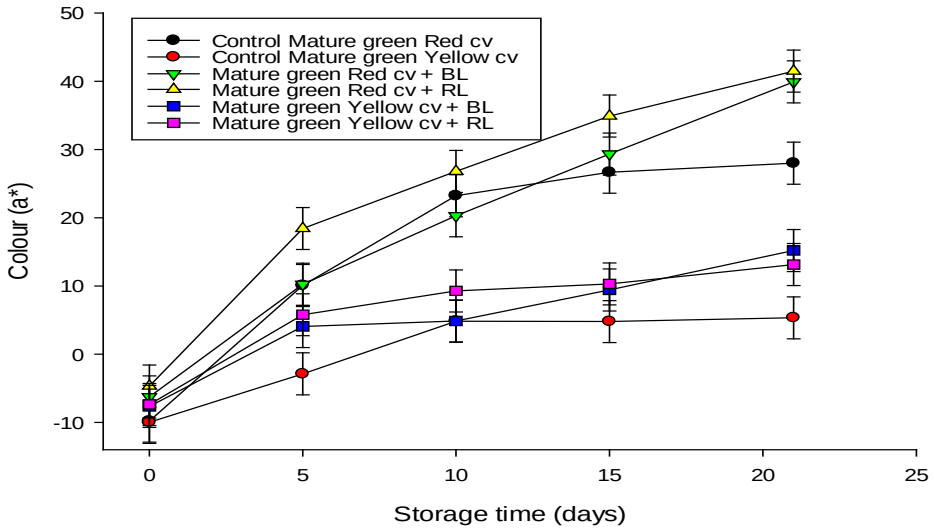

Figure 4.5: Red/green (a*) values of red and yellow cherry tomato fruit treated postharvest at the mature green stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 3.081].

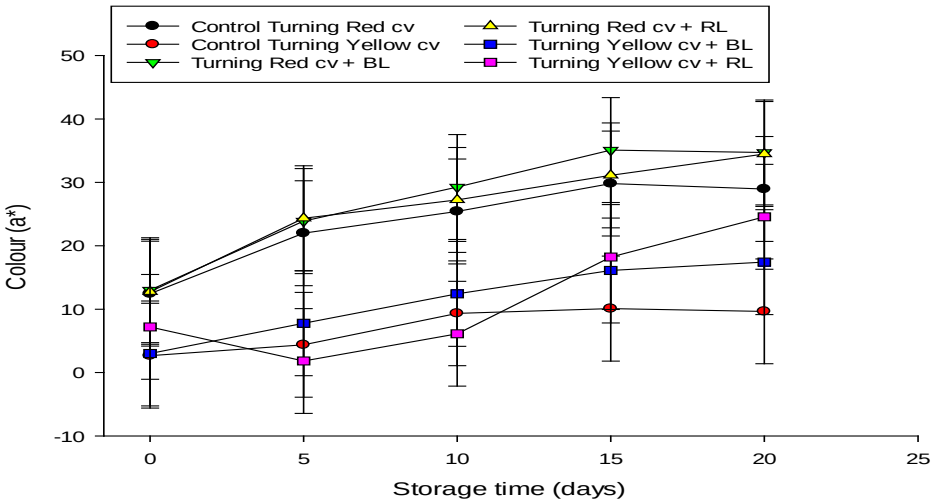

Figure 4.6: Red/green (a*) values of red and yellow cherry tomato fruit treated postharvest at the turning stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 8.272]

The rate of colour change from green to red of tomato fruit during postharvest storage did not seem to differ on the illuminated tomato fruits (P > 0.05). The control had a lower a* value from day 15 to the last day of storage (Figs 4.5 and 4.6); there was a significant difference between

the control treatment and other treatments at the mature green stage, while at the turning stage (Figs 4.5 and 4.6) there was no significant differences ($P > 0.05$).

The change in colour coordinate b* did not differ significantly between the stages ($P > 0.05$). Both red and blue lights were able to enhance the b* value of the yellow cv significantly (Figs 4.7 and 4.8). A steady decline in the change in b* value of the red cv was observed in both, tomatoes treated at the mature green and at the turning stage (Figs 4.7 and 4.8). No significant difference was observed between fruit treated at the mature green [LSD ($P_{0.05}$) = 7.768] and at the turning stages [LSD ($P_{0.05}$) = 7.027]. The b* value at the turning stage was initially higher than at the mature green stage, but at the last day of storage it was *vice versa*.

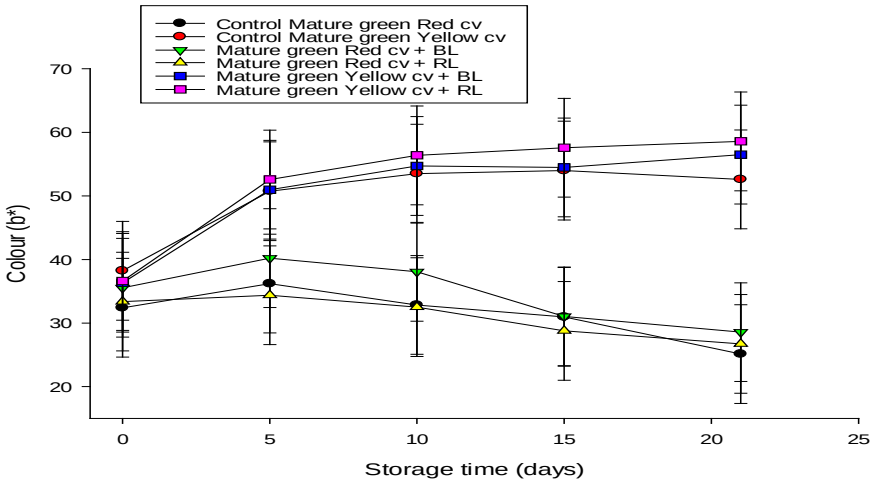

Figure 4.7: Yellow/blue value (b*) of red and yellow cvs of cherry tomato fruit treated postharvest at the mature green stage with various LEDs and stored at room temperature over a 21-day storage period [LSD ($P_{0.05}$) = 7.768]

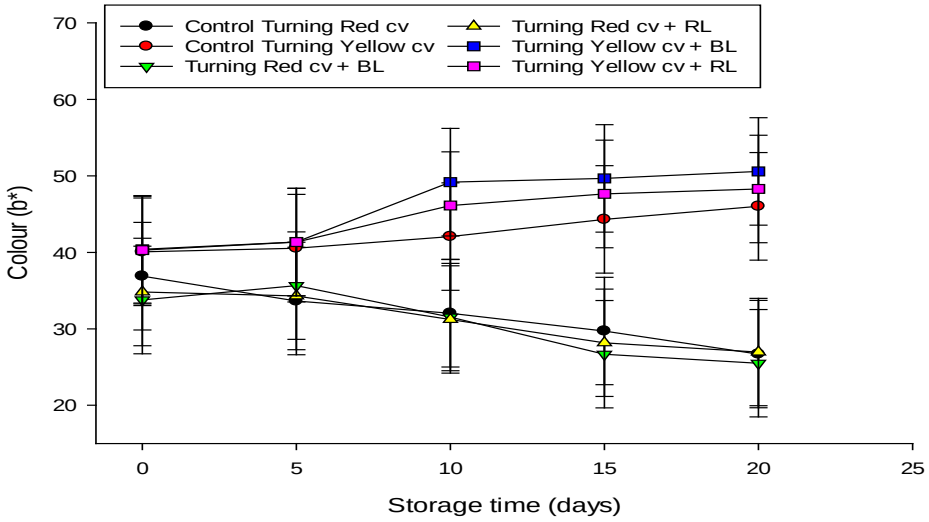

Figure 4.8: Alteration in yellow/blue value (b*) of red and yellow cvs of cherry tomato fruit treated at turning stage with various postharvest light treatments and stored at room temperature over a 21-day storage period [LSD ($P_{0.05}$) = 7.027]

4.5.3 Analyses of Pigments

Chlorophyll a, b and carotenoid concentrations in tomato differed significantly ($P < 0.01$) between treatments at both maturity stages; the control had the highest Chl a values until the last day of storage (Fig. 4.9). Both red and blue lights reduced the concentration of chlorophyll a athe faster than the control (Fig 4.9). A steady decline in chlorophyll a was observed in tomatoes treated at the turning stage. Light, however, did not have much effect on reducing the concentration of chlorophyll a at the turning stage (Fig. 4.10). The concentration of chlorophyll a was already low on day zero compared to the fruit treated at mature green. There was no significant difference between the treatments ($P > 0.05$) on fruit treated at turning stage (Fig. 4.10), while there was a significant difference on fruit treated at mature green stage ($P < 0.05$).

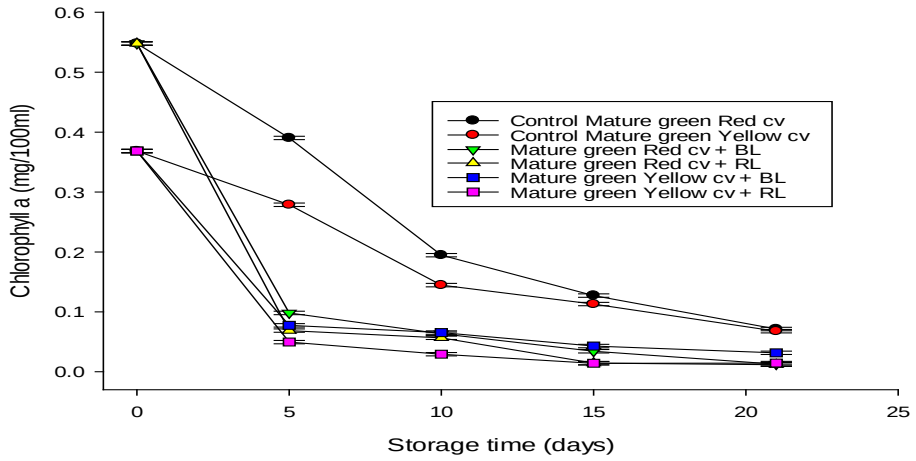

Figure 4.9: Chlorophyll a concentration (mg/100 ml) of red and yellow cvs of cherry tomato treated at mature green stage with various postharvest light treatments and stored at room temperature [LSD ($P_{0.05}$) = 0.003]

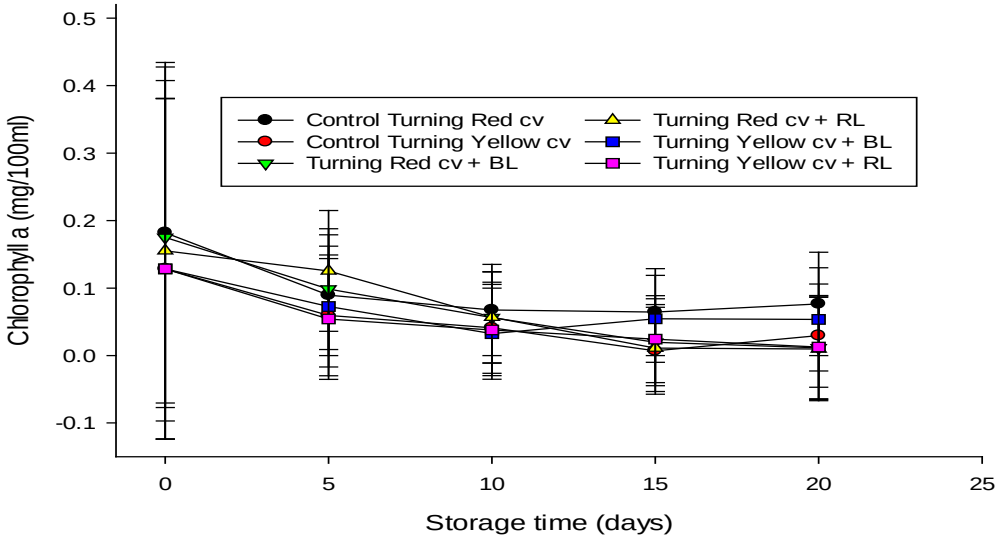

Figure 4.10: Chlorophyll a concentration (mg/100 ml) of red and yellow cherry tomatoes treated postharvest at the turning stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 0.001]

There was no significant effect of the light treatment on fruit at both maturity stages ($P > 0.05$) but there was a significant difference between the treatments ($P \leq 0.05$), with the green mature

control having the highest concentration of chlorophyll b from day 10 (Fig 4.11) and the turning stage fruit from day 15 (Fig. 4.12) onwards. A rapid decline in chlorophyll a was observed in all the treatments, except in the control (Fig. 4.11).

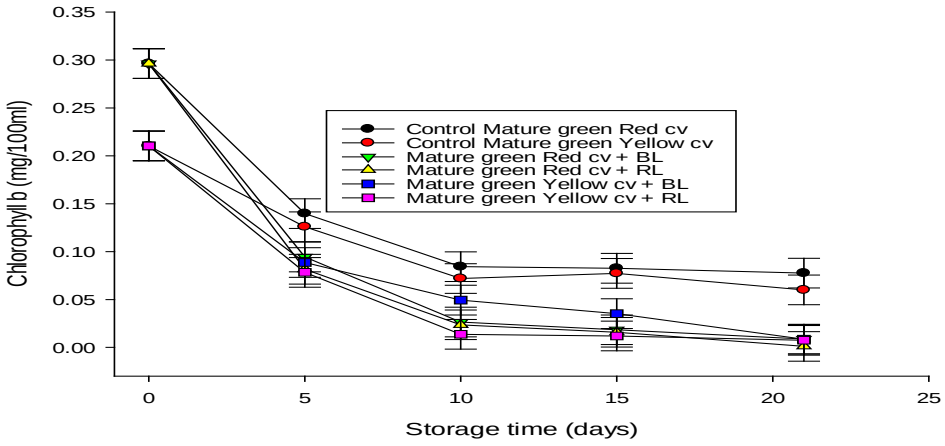

Figure 4.11: Chlorophyll b concentration (mg/100 ml) of red and yellow cherry tomatoes treated postharvest at the mature green stage with various LEDs and stored at room temperature [LSD ($P_{0.05}$) = 0.015]

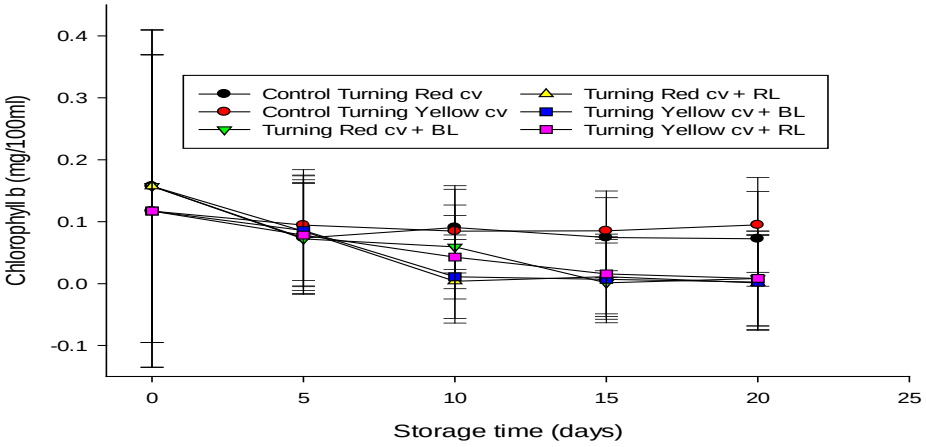

Figure 4.12: Chlorophyll b concentration (mg/100ml) of red and yellow cherry tomatoes treated at postharvest at the turning stage with various LEDs and stored at room temperature [LSD ($P_{0.05}$) = 0.0005]

A sharp increase in the lycopene concentration of the treated fruit was observed in the first two weeks for the red cv and thereafter a steady increase occurred (Fig. 4.13). Red light enhanced the concentration of lycopene more so than blue light in the red cv [LSD ($P_{0.05}$) = 0.019] (Fig. 4.13). Blue light had a lesser, but nonetheless significant effect on the accumulation of lycopene in yellow tomatoes. The concentration of lycopene in the yellow cv was low (0.2 mg/100 ml) (Figs 4.13 and 4.14). Fruit treated at the turning stage with light displayed a slower increase (Fig 4.14) in lycopene than those treated at the mature green stage. A significant difference between treatments was observed in fruit treated at the mature green stage ($P \leq 0.05$).

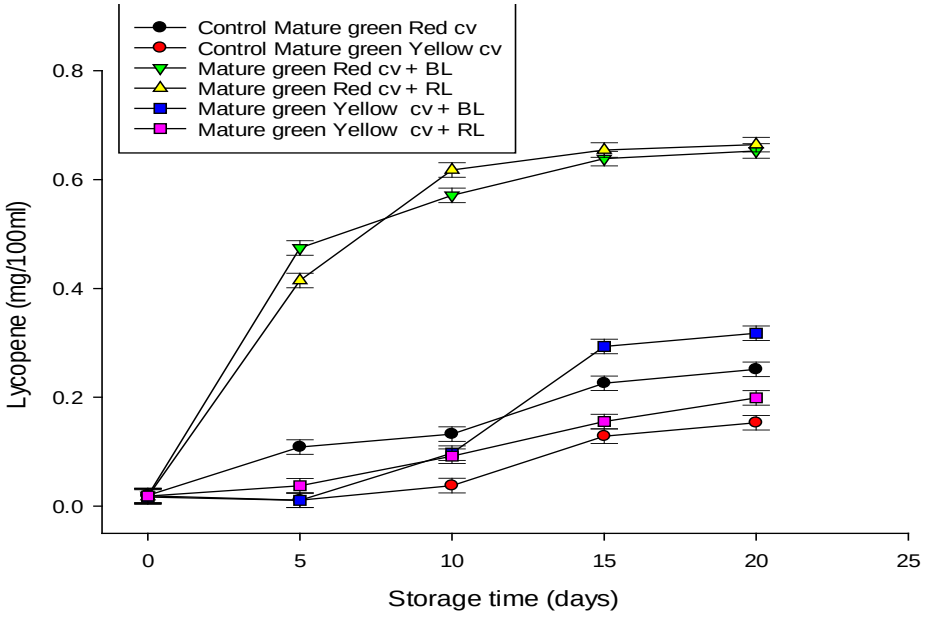

Figure 4.13: Lycopene concentration (mg/100 ml) of red and yellow cherry tomatoes treated postharvest at the mature green stage with various LED lights treatments and stored at room temperature [LSD ($P_{0.05}$) = 0.019]

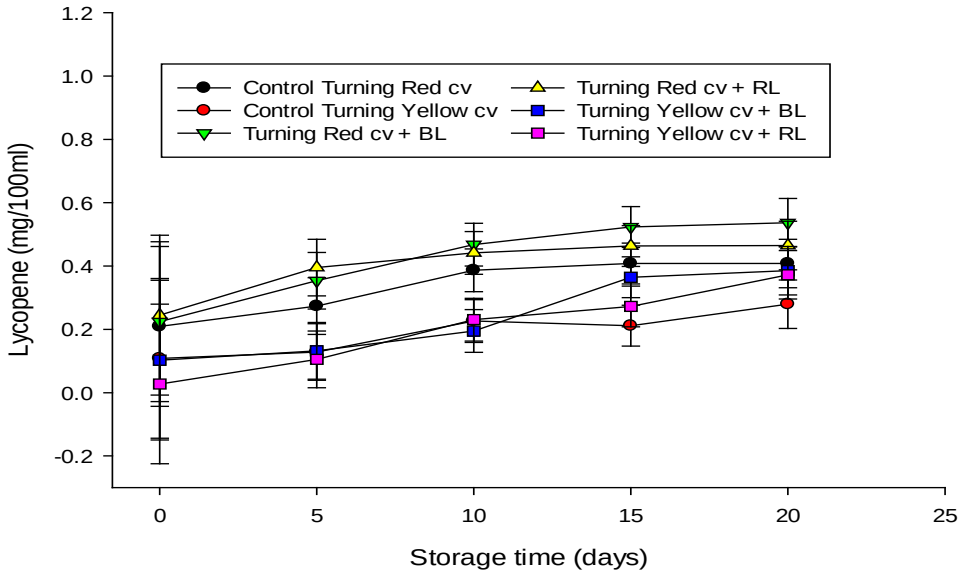

Figure 4.14: Lycopene concentration (mg/100 ml) of red and yellow cherry tomatoes treated postharvest at the turning stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 0.038]

A steady increase in the concentration of β-carotene was observed on tomatoes treated at the mature green stage and there were a significant differences between the treatments ($P < 0.05$) (Fig. 4.15). A rapid increase in the concentration of β-carotene was observed in fruit treated at the mature green stage. There was a significant increase between treatments from day 5 to the last day of storage [LSD (P0.05) = 0.047) (Fig 4.15]. In fruit treated at the turning stage, no significant difference between the light treatments and the control could be found ($P > 0.05$) (Figs 4.15 and 4.16).

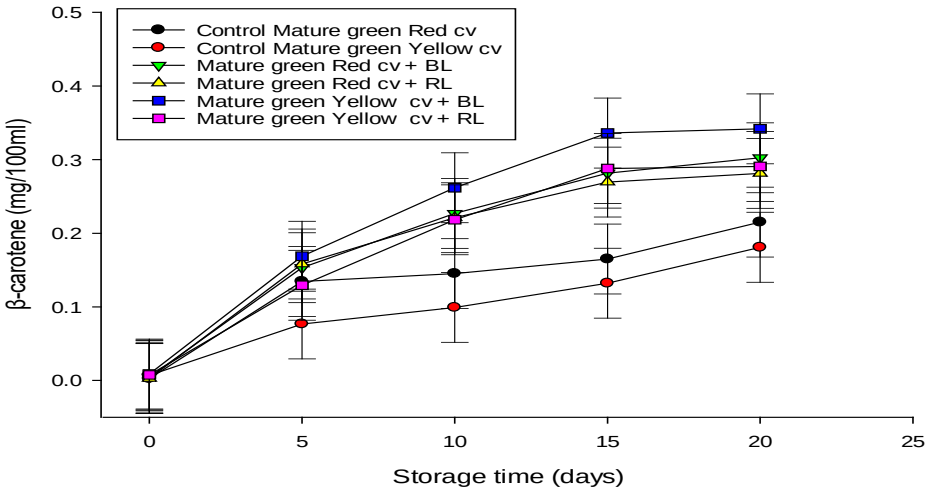

Figure 4.15: Beta-carotene concentration (mg/100 ml) of red and yellow cherry tomato fruit treated postharvest at the mature green stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 0.047]

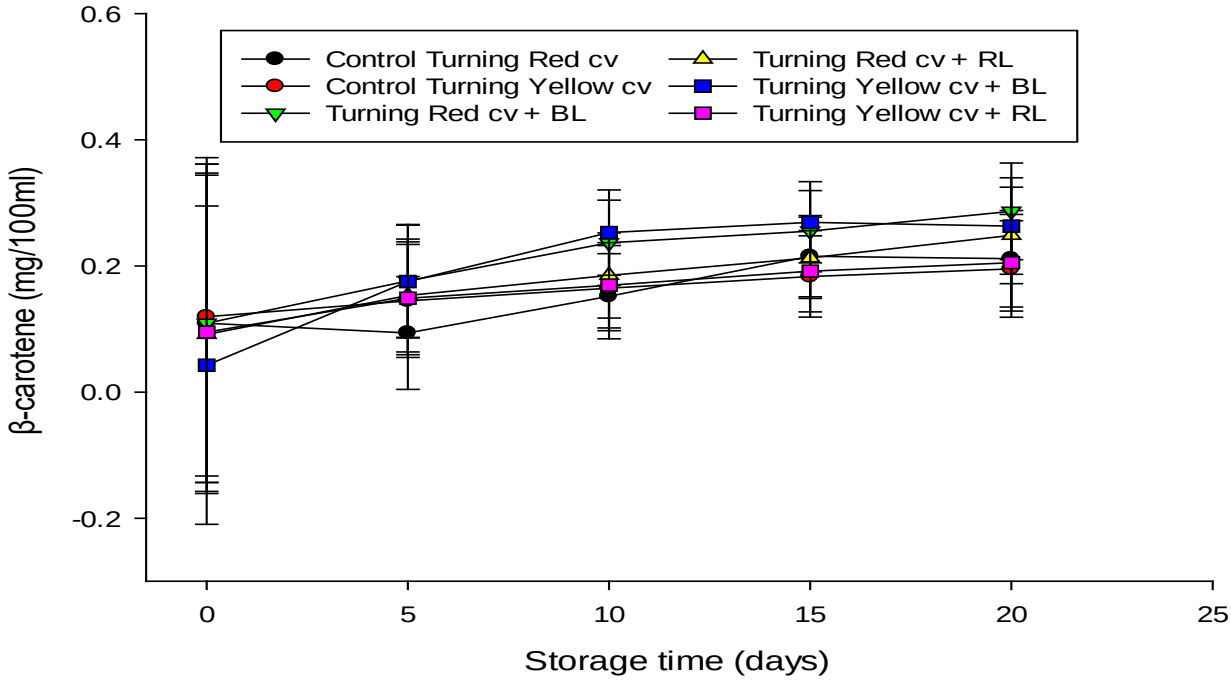

Figure 4.16: Beta-carotene concentration (mg/100 ml) of red and yellow cherry tomato cvs treated postharvest at the turning stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 0.038]

4.5.4 Total soluble solids

Light treatments did not have a significant effect on TSS ($P > 0.05$) at both maturity stages (Figs 4.17 and 4.18). In most treatments, TSS was higher than the control. Light increased TSS in fruit treated at the mature green stage (Fig. 4.17) more so than at the turning stage (Fig. 4.18). Red light had a significant effect on TSS for red cherry tomatoes treated at the mature green stage.

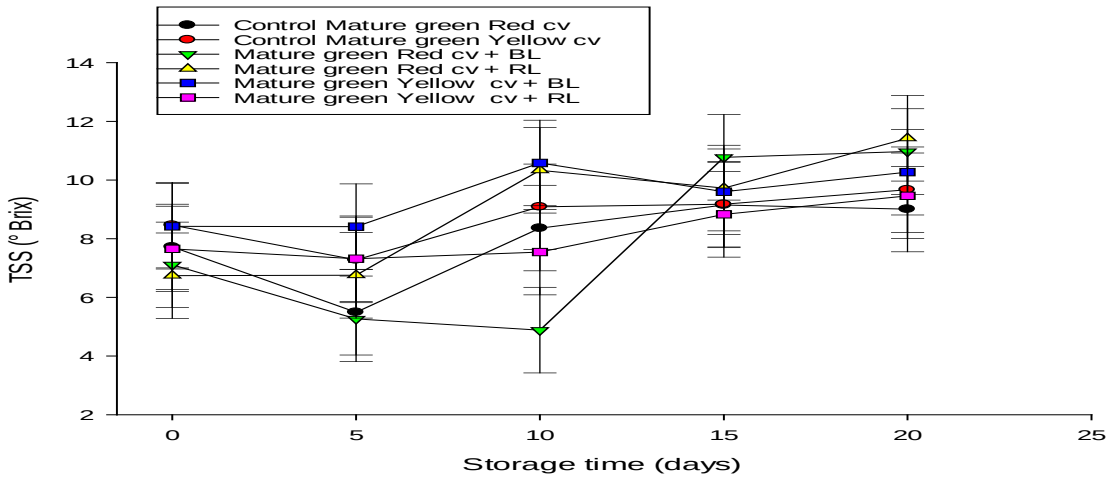

Figure 4.17: TSS (˚Brix) of red and yellow cherry tomatoes treated postharvest at the mature green stage with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 1.458]

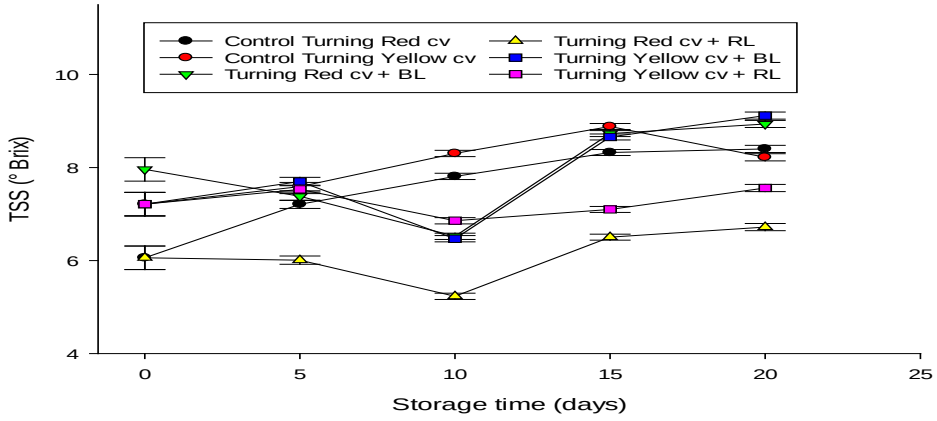

Figure 4.18: Changes in TSS (˚Brix) content of red and yellow cherry tomatoes treated at the turning stage with various postharvest light treatments and stored at room temperature [LSD ($P_{0.05}$) = 1.458]

4.5.5 Firmness

Initially all fruit treated with LEDs at the mature green stage (Fig. 4.19) had a tendency to be firmer, while fruit treated at the turning stage tended to be less firm (Fig. 4.20). After 5 days of treatment all fruit treated at the mature green stage still received a 'firm' rating, while those treated at the turning stage were already partially soft. On day 15, most treated fruit were 'partially soft' and some were already 'soft', while the control had a tendency to loose firmness slower. Fruit treated with red and blue lights were already soft on day 20. Some of the fruit evaluated on day 21 were partially soft. Light treatment therefore resulted in softer fruit when treated at the mature green [LSD ($P_{0.05}$) = 0.601] or at the turning stage [LSD ($P_{0.05}$) =0.579].

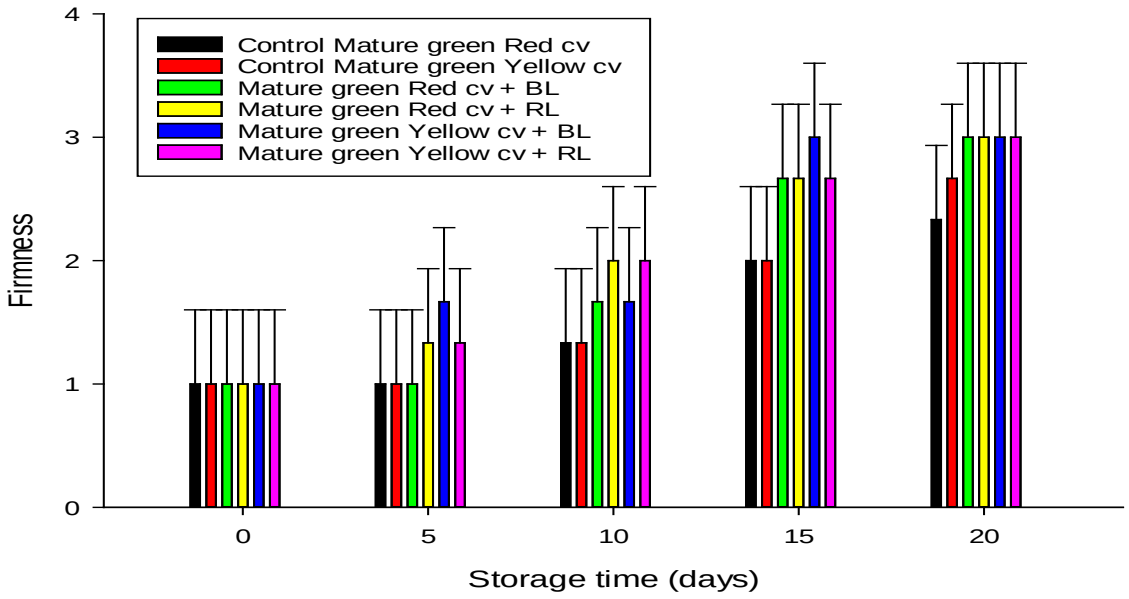

Figure 4.19: Manual firmness evaluation of red and yellow cherry tomato cvs treated post-harvest at the mature green with various LED lights and stored at room temperature [LSD ($P_{0.05}$) = 0.601].

The values 1 (firm), 2 (partially soft) and 3 (soft) were used to rate the firmness of cherry tomatoes.

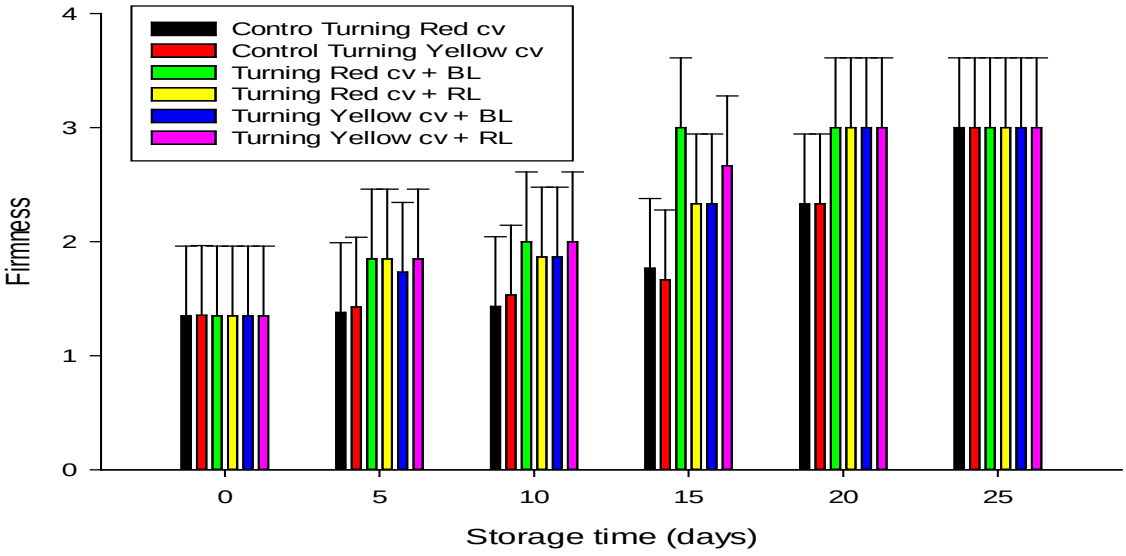

Figure 4.20: Manual firmness evaluation of red and yellow cherry tomato cvs treated postharvest at the turning stage with various LED lights and stored at room temperature (LSD ($P_{0.05}$) =0.579)

There was also no significant difference between the treatments (P >0.05). The values 1 (firm), 2 (partially soft) and 3 (soft) were used to rate the firmness of cherry tomatoes.

4.6 DISCUSSION

The use of post-harvest light treatments affected quality attributes of the two tested cultivars of cherry tomato (*Solanum lycopersicum*); the red 'Cherry Little Wonders'), however, responded differently to the LED treatment than the yellow one ('Goldilocks'). Additionally, the response also depended on the stage of the fruit maturity at which the light treatment was administered. As a climacteric fruit, tomato displays an increase in respiration during ripening (Chalmers and Rowan, 1971); this resulted in a fruit mass loss (Figs 4.1 and 4.2). The increase in temperature in the environment due to respiration causes the metabolic rate to be accelerated resulting in acceleration of water loss from the fruit, the primary reason for the reduction in fruit mass (Mutari and Debbie, 2011). Postharvest blue and red light treatments affected mass loss of cherry tomatoes at the mature green stage in a different way to fruit at the turning stage. Fruit that

received blue light at the mature green stage lost about 0.004% mass under both wavelengths and in both the yellow and the red cultivars. Fruit that received red light, on the other hand, lost less mass than those that received blue light (Fig. 4.1). At turning stage (Fig. 4.2) there was no statistical difference between the light treatments [LSD ($P_{0.05}$) = 0.0005] and this may be because the fruit respired more at the green mature stage when treated with BL than RL. From day 10 fruit were no longer losing water, as no change in mass loss could be recorded. Light affected red and yellow cherry tomatoes in the same way, since no statistical differences were observed ($P > 0.05$). Due to LEDs not emitting heat like fluorescent or incandescent light, this stationary mass, could indicate that fruit had completed the respiratory peak and mass loss was now too little to be perceived on a daily basis. Light emitting diodes (LEDs) emit low emission of radiant heat which lowers the harmful effects of radiant heat on the quality of agricultural commodities (Morrow, 2008; Mitchell et al., 2012).

Mass loss in fresh vegetables and fruit causes a shortening of the shelf life coinciding with a loss in economic value of the commodity (Kraśniewska et al., 2014). It is likely that the increase in mass loss of cherry tomatoes was not associated with light effects, as there was no significant difference ($P < 0.05$) in mass loss of fruit that received either red or blue light. This was similar to non-treated fruit, meaning the LED treatment did not have any negative effect on tomato fruit treated at the mature green stage and the turning stage. These results are in line with findings of Smock (1977), who reported that high temperatures around the fruit and long duration of exposure to such temperatures are key factors of rapid mass loss. Storage period and treatments had a statistical significant ($P < 0.05$) effect on mass loss, confirming earlier results by Lurie and Sabehat (1997) who worked on standard tomatoes (*Solanum lycopersicum* cv. Daniella), as well as results by Artes et al., (1998) who worked on red raspberry jam (*Rubus pubescens*) and Javanmardi and Kubota (2006) who worked with red stage ripened cluster tomatoes (*Solanum lycopersicum* Mill. cv. Clermon).

All fruit were packed into micro-perforated packages, allowing a reduction in fruit mass (Choi et al., 2015). It is believed that fruit would have lost much more mass if these packages had not been used. Micro-perforated packaging is also useful to avoid postharvest fruit ripening and associated biochemical and physiological changes by favorably altering the O_2 and CO_2 levels inside the package. The combined effects of ultraviolet-C (UV-C) irradiation and modified

atmosphere packaging (MAP) was investigated on inoculated Typhimurium (*Salmonella enterica* serovar) and non-inoculated cherry tomatoes. The results suggested that the combination of UV-C irradiation and MAP can improve the microbial safety and extend the shelf life of cherry tomatoes during storage (Choi et al., 2015). Koide and Shi (2007) reported that a mass loss greater than 5% causes a reduction in the retail value of fresh produce. Javanmardi and Kubota (2006) reported that higher rates of transpiration and respiration in tomatoes stored at 25-27°C compared with tomatoes stored at 5-12°C could be the main factor for increased rates of mass loss in warmer environments. Thanh (2006) indicated that storage temperatures above 20°C can result in abnormal physiological processes in fresh produce. These results confirm that postharvest environmental conditions, including storage temperature and packaging need to be considered carefully to ensure the physicochemical quality of cherry tomatoes during storage. In the study, no mass loss exceeding 5% was observed, meaning that the use of micro-perforated packages allowed a minimal reduction in mass loss.

Firmness of cherry tomatoes decreases during storage. The fruit becomes soft due to metabolic changes induced by enzyme action. These enzymes have been identified as the hydrolases polygalacturonase (PG) and pectin methylesterase (PME) (King and O'Donoghue, 1995; Sethu et al., 1996); however, other enzymes, such as xylanase and glycosidase (Campbell et al., 1990) and cellulase (Awad and Young, 1979), also play important roles in fruit softening. These enzymes seem not to have been affected by the light treatment, as no significant difference in softness was found between treatments (Figs 4.19 and 4.20) ($P > 0.05$). In a study by Gharezi et al. (2012) all fruit softened progressively during storage. Firmness of tomato was influenced by temperature and storage time, decreasing during storage. This finding is of great importance, as, although colour is the primary factor of attracting the consumer to ripe tomato fruit, firmness is likely to be the factor on which the purchasing decision is made (Pinheiro et al., 2013). Softening of tomato fruit is of great economic importance for both producers and consumers, as softer fruit have a lower market value because of lower consumer acceptance (Gastélum-Barrios et al., 2011; Pinheiro et al., 2013). Treatment with red and blue LEDs did not stop but rather had a tendency to accelerate the loss in firmness of tomato fruit (Figs 4.19 and 4.20).

There was no statistically significant difference ($P> 0.05$) in internal quality parameters of fruit treated with red and blue lights at the mature green or the turning stage and the control with

regard to firmness. Initially, all fruit that had received light treatment at the mature green stage were 'firm' (Fig. 4.19); after day five they still received the 'firm' rating and the reduction in firmness did not differ significantly between treatments. On day 20, most fruit were partially soft and on day 25 all fruit were soft. Only the control had a tendency to be still partially soft. The fruit subjected to light at the green mature stage were getting closer to receive a 'partially soft', while a significant loss in firmness was observed on the fruit treated at the turning stage. Light effects on firmness did not differ in this study; however, Dhakal and Baek (2014) reported that irradiating tomato fruit at the mature green stage with blue light (at 440-450 nm) results in firmer fruit than subjecting them to red light (650-660 nm). Decay and incidences of diseases was minimized by a combination LEDs and MAP. In addition, results indicate that LED or MAP affect firmness of the cherry tomato fruit to a lesser extent than storage temperature. Fagundes et al. (2014) reported that fruit softening is triggered by biochemical processes, involving the hydrolysis of pectin and starch in the cell wall; these enzymes are predominantly pectin methyl esterase and polygalacturonase. The use of light treatment stimulated the biochemical ripening processes of cherry tomato, as a result the fruit became softer. The higher the temperature or the longer the duration the fruit is subjected to higher temperature, the quicker the biochemical processes in the fruit occur, causing the fruit to soften quickly.

As the fruit become softer, the surface colour changes; colour is a human perception and has long been used in the assessment of fruit quality. As the concentration of chlorophylls decreases, the fruit becomes softer, loses mass and becomes more mature. These phenomena are traditionally used as the criteria for visual assessment of fruit maturity (Valero et al., 2007). The various sequences of colour change observed in different cultivars of cherry tomato fruit treated with different treatments (Figs 4.5 to 4.8), are likely to be the result of the various rates of chlorophyll degradation and carotenoid formation. Tomato fruit treated with red LED light seemingly had a higher rate of carotenoid synthesis and chlorophyll breakdown. This could explain the faster conversion from green to red/yellow colour of cherry tomatoes following light treatment.

In order to assess ripeness and postharvest life of tomatoes, colour of tomatoes fruit is the most vital sensory characteristic and the consumers' purchase decision relies, firstly, on fruit colour. During ripening the colour of tomatoes changes and these changes are the result of two

simultaneous processes: Firstly, the degradation of chlorophyll to a colourless, but fluorescent chlorophyll catabolite and ultimately to a non-fluoresecnt chlorophyll catabolite occurs (Hörtensteiner and Kräutler, 2011). Secondly, carotenoids are synthesized from a colourless precursor (phytoene) to lycopene (red), β-carotene (orange), xanthophylls and hydroxylated carotenoids (yellow) (Radzevičius et al., 2009). The mean *a** value of mature green cherry tomatoes was around -10 to -9 initially (Fig. 4.5). After 25 days, fruit that were treated with light at the mature green stage had a higher *a** value than the control. Similarly, in fruit treated at the turning stage the *a** value increased, but the fruit treated when mature green were redder at experiment termination, with an a* value of 43, than those treated at the turning stage (a* value of 33). There was no significant difference between the effect of red and blue LED lights on red cherry tomato fruit at both maturity stages. Light treatments hardly enhanced the colour of tomatoes treated at the turning stage. The yellow cultivar had a lower a* value at both stages; however, the value of b*(green to yellow) was high in the yellow cv from day 10 to day 21. A rapid (Fig. 4.7) and steady (Fig. 4.8) change in color (b*) was observed in tomatoes treated at the turning and mature green stage, respectively. It is worth noting that the high *a** value for treated tomatoes was due to the loss of chlorophyll and accumulation of carotenoids as a result of the ripening process. Similar results were obtained by Liu et al. (2009), who studied the effects of UV-C, red light and sunlight on the carotenoid content and physical qualities of tomatoes postharvest. The authors found that the colour (*a** and *b** values) and force required to penetrate the tomatoes was, to a small but significant extent, influenced by red light treatments resulting in the accumulation of carotenoids. A study by Li and Kubota (2009) revealed that the concentration of phytochemicals of baby lettuce can be increased by blue light. Dhakal and Baek (2014) also reported that colour development of tomatoes and pepper can be enhanced by red light (at 650-660 nm).

A significant statistical difference in luminosity (L* value) was observed between treated and non-treated cherry tomatoes [LSD ($P_{0.05}$) = 3.793, Fig 4.3]; [LSD ($P_{0.05}$) = 4.883, Fig 4.4]. Liu et al. (2009) obtained similar results. These authors found that the L* value decreased significantly in tomatoes treated with red light as the fruit changed colour from green to red/yellow. The change in L* did not differ significantly between the cultivars and the type of light used ($P < 0.05$).

According to Johnson et al. (2002) reactions in the formation of pigments depend on the metabolic energy provided by ATP. It seems probable that fruit receiving additional light energy through the LEDs made use of this additional energy to increase the rate of pigment forming reactions, which enabled faster appearance of pigments in illuminated tomatoes. The reaction will be faster in the presence of the activation energy on the condition that concentration of the substrate is not limited. The faster rate and faster complete colour change in cherry tomatoes treated with light could be explained by the light-dependent synthesis of some enzymes, especially those involved in the formation of red carotenoids.

Most fruit, including tomato, show a sharp decrease in chlorophyll concentration and a corresponding increase in carotenoid synthesis during the fruit ripening process. This change is as a result of the conversion of chloroplasts into chromoplasts. Figs 4.9 to 4.12 depict that the chlorophyll concentration of treated and control cherry tomato fruit decreased during storage. The chlorophyll concentration of control fruit was higher than that of fruit in other treatments until day 25, the last day of storage. A significant difference ($P \leq 0.05$) among treatments in fruit chlorophyll content was observed after day 14. The chlorophyll concentration was significantly higher, initially, and decreased after receiving light treatment. This implies that fruit without light treatment showed a slower degradation of chlorophyll, while control fruit retained more chlorophyll than treated fruit (Figs 4.9 to 4.12). Similar results were obtained in the study by Tadesse and Abtew (2016) who determined the effect of hot water and light treatments on quality attributes of fresh tomatoes.

A statistical significant difference ($P \leq 0.05$) in the concentration of lycopene and ß-carotene was observed between treated and control cherry tomato fruit. In fruit receiving light treatment, carotenoid accumulation seemed to have been higher than in the untreated fruit (Figs 4.9 to 4.12). A significant decrease in chlorophyll concentration in fruit treated with red and blue lights was accompanied by biosynthesis of lycopene and β-carotene. Chlorophyll degradation and lycopene accumulation, which are the most important colour-affecting processes during fruit ripening and senescence, commenced after a few days of light treatment. Chlorophyll destruction and accumulation of carotenoids and lycopene was aligned with the generation of the normal red colour in ripening fruit. Red fruit treated at the mature green stage had a high concentration of lycopene (Fig. 4.13) combined with a rapid degradation of chlorophylls a and b (Figs 4.9 and

4.12) compared with those treated at the turning stage. Red light had a significant effect on the accumulation of lycopene at both stages of development, but no statistical difference between red and blue lights existed in terms of carotenoid accumulation. These results are in accordance with Dhakal and Baek (2014) who reported that LED light wavelengths of 650-660 nm increased the concentration of lycopene in mature green tomato fruit.

Yellow tomatoes had a very low lycopene concentration at both stages of green mature and ripe (Figs 4.13 and 4.14). This is not surprising, as lycopene is associated with the red pigment of tomatoes. This does, however, not mean that yellow tomatoes do not contain lycopene, but do so at a reduced rate (Rego et al., 1999). Other yellow carotenoids are also present in yellow tomato, such as β-carotene and lutein (Fantini et al., 2013). Yellow tomatoes contain little to no lycopene but contain other carotenoids with a potentially even greater antioxidant capacity than red tomato (Shi and Maguer, 2000). Red and yellow tomatoes contain different forms of lycopene (red tomatoes contain trans-lycopene and yellow tomatoes contain tetra-cis-lycopene). The antioxidant is equally potent in both forms but the human body easily accesses lycopene in the cis-form (Giovannucci, 1999; Shi and Maguer, 2000). While no significant difference between treatments was observed in carotenoids of fruit when treated at the turning stage, a steady increase in β-carotene was observed within the first eight days, but thereafter no further β-carotene accumulation could be detected. The opposite was, however, observed in the fruit treated at the mature green stage. Gangadhar et al. (2012) demonstrated that chlorophylls can be enhanced by blue LED light and carotenoids can be enhanced by the combination of red and blue LED lights in chilli pepper fruit.

While carotenoids are beneficial to human health, commercially total soluble solids (TSS) (expressed as °Brix) is used in tomatoes as an indicator of fruit quality, since it is aligned with fruit sweetness. On the fruit harvesting day °Brix values of the fruit varied, ranging from 6-8 °Brix and were not significantly ($P > 0.05$) influenced by any of the light treatments. In addition, no significant ($P > 0.05$) changes in TSS values were detected in both untreated and light-treated samples during storage, with values remaining within the range of 8 and 9 °Brix. At first, °Brix values decreased in all treated fruit (Figs 4.17 and 4.18) and later, after 15 days, they started to increase again. There was no statistically significant difference between treated and untreated fruit at both stages of development in terms of TSS. Liu et al. (2009) also reported similar results

after exposing mature green tomatoes to short bursts of UV-C light (1.37 J/cm^2, 5 min) for up 21 days. Light did not dramatically influence the total soluble refractive solids of tomatoes. Tomato sugars contributes to fruit flavor and total soluble solids are predominantly sugars. In general, the flavour of a fruit becomes pronounced when the sugar content peaks. The sugar content of tomatoes depends on the stage of maturity. It increases uniformly from immature to green mature to red-ripe tomatoes (Javanmardi and Kubota, 2006).

According to Manurakchinakorn et al. (2014) heat and light treatments that increase chilling tolerance is believed to act via induced synthesis and accumulation of specific heat-shock proteins (HSPs). In the study there were no major symptoms of chilling injury or physiological disorders and also no decay was noticed. Similar results were also obtained in the study by Zhang (2005).

4.7 CONCLUSIONS

The present study showed that treatment of cherry tomatoes with red and blue lights enhances the production of lycopene and carotenoids in general. The stage at which fruit are exposed to the treatment influenced the accumulation of lycopene and ß-carotene, with the mature green stage being more sensitive to the light treatment than the turning stage. This indicates that exposing tomato fruit at the mature green stage alters fruit pigments and colour more effectively than applying LED lights at the turning stage. The results of this study correspond with the results of Tadesse and Abtew (2016) and Yang et al. (2009). Moreover, the failure of control fruit to ripen properly (accumulation of lycopene and carotenoids) could be the result of interruption in the conversion of chloroplasts to chromoplasts due to the destruction of plastids. Changes in the quality parameters of red and yellow cherry tomato fruit could be kept within the determined ranges by combining storage conditions with red or blue light and MAP treatments. Disorders and decay were reduced following light treatments. Therefore, treatments prevented overall quality loss. In conclusion, the fruit of red cherry tomato treated with red light at the mature green stage was the most effective treatment, as all quality attributes of the fruit were maintained; however, no significant difference was observed between treatments with red or blue lights.

Further research needs to evaluate the effect of these light sources and MAP individually and in combination on the quality attributes of different tomato cultivars at different stages of development. Future research is also needed to find the treatment that can enhance the accumulation of lutein, one of the most powerful carotenoids in yellow tomatoes.

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CHAPTER FIVE

GENERAL DISCUSSION AND RECOMMENDATIONS

Pre- and post-harvest red and blue light treatments were administered to two cherry tomatoes (*Solanum lycopersicum*), the red cultivar 'Cherry Little Wonders' and the yellow cultivar 'Goldilocks'. Two experiments were conducted, one in the glasshouse and another one in the post-harvest laboratory at the University of KwaZulu-Natal in 2017. The first experiment was designed to evaluate the effect of pre-harvest red and blue light treatments on colour, ripening, chlorophyll and carotenoid concentration as well as overall quality of the two cherry tomato cultivars. The second experiment compared the post-harvest effect of red and blue light treatments on colour, ripening, carotenoids and quality of the two cultivars treated at different fruit ripening stages.

5.1 PRE-HARVEST ALTERATIONS IN TOMATO FRUIT QUALITY FOLLOWING EXPOSURE TO RED AND BLUE LED LIGHTS

In this study, pre-harvest red and blue lights significantly affected the measured quality attributes of the red and the yellow tomato cultivars. Red and blue lights had no negative effect on fruit size, but affected colour and pigments significantly.

Sugars in tomato accumulates during ripening but that depends on the cultivar and the treatments involved. Sugars contributes to flavor and total soluble solids (which are predominantly sugars). Sugars and TSS were not significantly affected by red and blue lights.

Tomato fruit subjected to different treatments displayed various sequences of colour change, which could be the result of various rates of chlorophyll degradation and carotenoid formation. Both light treatments enhanced colour change in both red and yellow cultivars of cherry tomatoes. Colour is the first characteristic that determines the degree of consumer's acceptance, while the final quality parameter consumers judge tomatoes on is firmness; this parameter ultimately makes the consumer decides to buy fresh tomatoes. Firmness of cherry tomatoes was

associated with concentration of chlorophylls and that was correlated with increasing maturity; this is traditionally used as the criterion for visual assessment of fruit maturity. Both red and blue lights affected firmness of cherry tomatoes, however there was no significant difference in softness between treatments and the control.

Light treatments did not only affect colour, size and pigments, but it was able to prevent spoilage associated with diseases on tomatoes. There were no symptoms of diseases, physiological disorders and chilling injuries in the treated fruit compared to the control.

5.2 POST-HARVEST ALTERATIONS IN TOMATO FRUIT QUALITY AT DIFFERENT STAGES FOLLOWING EXPOSURE TO RED AND BLUE LED LIGHTS

The response of tomato fruit that received post-harvest light treatments did not differ significantly with the fruit that was treated and allowed to ripen on the tree. Light treatments were able to enhance colour development more on cherry tomato fruits treated at mature green compared to those treated at turning stage.

As the colour changes in tomato fruit, it losses water and chlorophyll degradation occurred, accompanied by accumulation or biosynthesis of pigments and carotenoids. The effect of light on chlorophylls a and b on fruit varied according to the cultivars. Fruit that were treated at turning stage had lower chlorophylls initially and then a steady rate of change was observed while a sharp/rapid degradation of chlorophylls was observed in fruit treated at mature green. Light effects on degradation of chlorophylls had no significant difference within the stage at which plants received light. Lycopene was the major pigment in red cv of cherry tomatoes. It was influenced equally by red and blue lights. Fruit treated at mature green had more lycopene than those treated at the turning stage. There was a significant difference between treatments and the control in terms of lycopene and β-carotene contents which were higher in fruits treated at mature green stage.

There was no significant difference ($P < 0.05$) in change in mass of fruit that received red and blue light and non-treated fruit, meaning that lights did not have a negative effect on tomato fruit treated at mature green stage and at the turning stage.

5.3 RECOMMENDATIONS

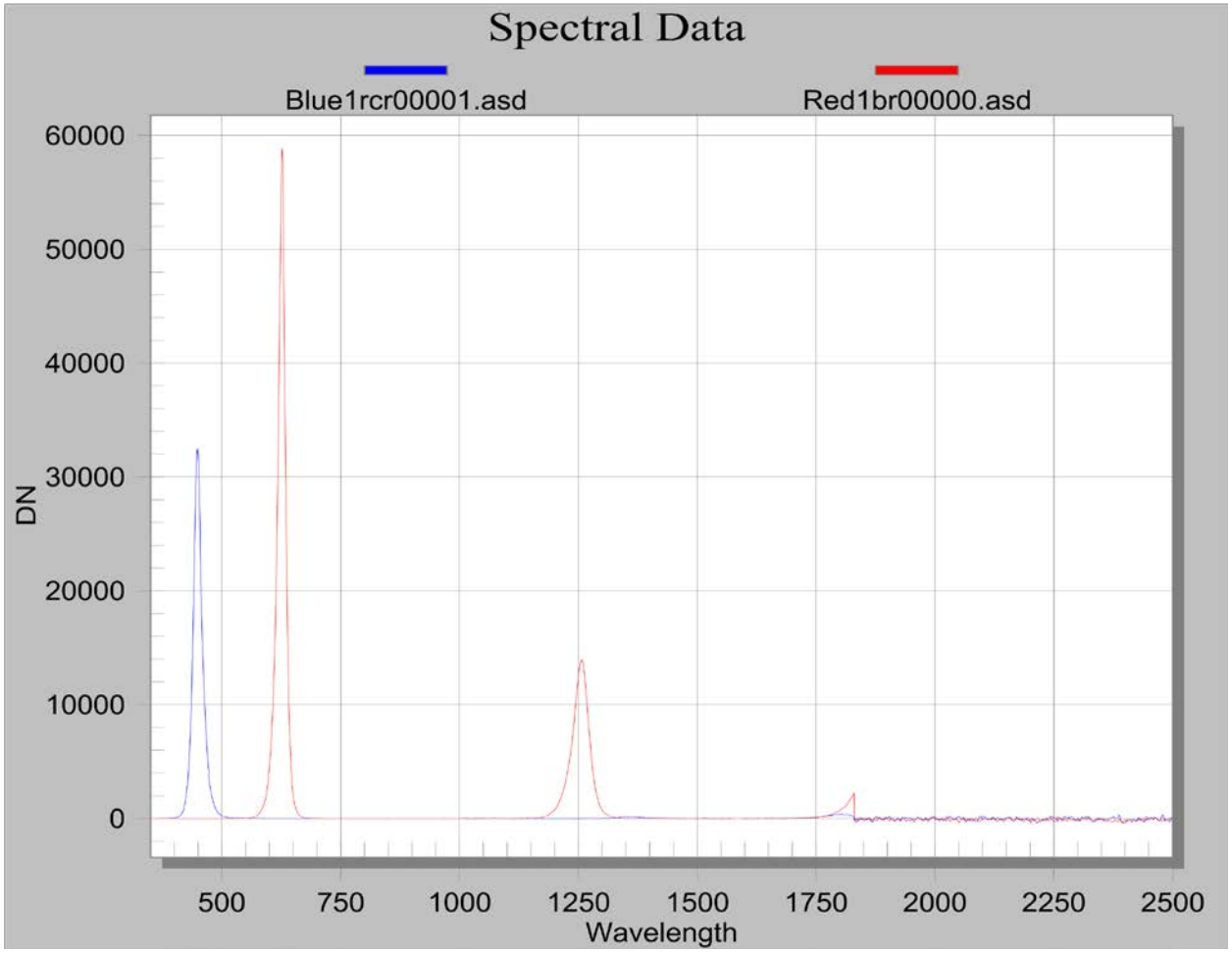
- It is recommended to treat cherry tomato fruit, while still attached to the mother plant because fruit still continued increasing in size, making the final product more marketable and giving better returns. Treated fruit had a higher concentration of lycopene and β-carotene, colour development was also enhanced which resulted to short ripening period.
- Treating tomato fruits at mature green stage with post-harvest light effect could enhance colour development and more pigments and carotenoids with less effect on mass loss. Therefore, it is recommended to harvest tomato fruit and subject them to light at the mature green stage.
- Both blue and red light treatments had positive effects on colour development, synthesis of various phytochemicals and other quality parameters, however, red light should be preferentially used as it enhanced lycopene and increased yield of tomatoes while blue light did not improve fruit size.
- Red cv (Cherry Little Wonders) of cherry tomatoes had high content of both lycopene and β-carotene, while yellow cv (Goldilocks) had only high content of β-carotene but very low lycopene. Therefore, it is recommended for the consumer to eat both red and yellow cherry tomatoes so as to get all the desired carotenoids in tomato.

5.4 SCOPE AND FUTURE RESEARCH

- More experiments should be carried out to determine the role of various LED lights (different intensities) treated individually and in combination on various cultivars of cherry tomato.
- Future research needs to be done to find the treatment that would be able to enhance lycopene in yellow cvs of cherry tomato.
- Future research should be carried out to determine the various carotenoid content present in the tomato fruit and if the various pigments respond differently to light treatment.

- Future research should be carried out to determine the effect of light treatment on fruit vitamin C content and antioxidants in the fruit.
- The pigmentation in the yellow cultivar needs further investigation.
- It also needs to be investigated if lengthening/ shortening the period of light exposure would alter the response.

Appendix A



Spectral characteristics of lamps used as a supplemental lighting in two cultivars (Cherry Little Wonders and Goldilocks) of red and yellow cherry tomatoes (*Solanum lycopersicum*) during greenhouse cultivation. Light treatments: red (R) and blue (B) LED.