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The role of post-harvest supplementary light exposure for ripening and quality development of tomato fruit

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Referent:	**Herr Prof. Dr. Georg Noga**
Korreferent 1:	**Herr Prof. Dr. Andreas Schieber**
Korreferent 2:	**Herr Prof. Dr. Peter Schulze Lammers**
Korreferent 3:	**Herr PD Dr. Maurício Hunsche**

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The role of post-harvest supplementary light exposure for ripening and quality development of tomato fruit

The major aim of the present work was to evaluate the optimal exposure time and effects of red light and short periods of daily ultraviolet (UV) on the post-harvest quality of green tomato fruit during ripening. The concomitant objective was to work out fundamental knowledge about the mechanisms of secondary metabolite compounds in lab-scale of post-harvest tomato under optimal light condition. For this purpose, experiments were conducted in a climate chamber equipped with red light-emitting diodes (LED) and UV tubes. The studies on green stage-1 tomatoes include investigations on the impact of light exposure on level of external fruit color Normalised Difference Vegetation Index (NDVI), Normalised Anthocyanin Index (NAI), Simple Chlorophyll Fluorescence ratio (SFR_R), firmness and total soluble solid content after post-harvest. To prove suitability of the light to track change in secondary metabolite compounds, lycopene and β-carotene concentration, determined by (means of) HPLC analyses. Total phenolic, and total flavonoid concentrations and antioxidant activity were analyzed by spectrophotometric detection. Here again, the fluorescence-based indices were compared to the well-established reflection-based ripening index a*/b*. The results ascertained in the single chapters can be summarized as follows:

1. Green tomatoes were exposed for 30 min to UV radiation, continuous red light or a combination of both for up to 20 d. Non-treated (control) fruits ripened within 15 d while fruits exposed to red light and a combination of red light with UV radiation required five days less to reach the same maturity level. Moreover, the exposure to red light alone or in combination with UV raised concentrations of lycopene, ß-carotene, total flavonoids and phenolics. This possibility to steer the concentrations of health promoting antioxidants through light treatments is a reliable method to increase fruit quality according to customer wishes and demands.
2. The impact of post-harvest red light irradiation via LED modules on the content of health promoting compounds was evaluated on green tomatoes stage 1 exposed to intermittent or continuous irradiation. The first experiment studied the overall effects of different duration periods of red light radiation (darkness, continuous red light for 10 d, continuous red light for 15 d and continuous red light for 20 d) while the second experiment focused on effects of intermittent red light (darkness, red light for 30 min per day, red light for 6 h per day, red light for 12 h per day and continuous red light). In both experiments, tomatoes exposed to darkness served as control. Continuous red light irradiation accelerated the ripening process of green tomatoes. In addition, continuous red light also significantly increased lycopene, β-carotene, total phenolic content, total flavonoid concentration and antioxidant activity compared to all other treatments, suggesting that continuous red light exposure positively influences metabolic processes and contributes to a higher content of health promoting compounds in tomatoes.
3. In the last section, green stage-1 tomatoes were harvested and treated daily with red light for 12 hours per day, for 15 days (followed by storage in darkness for additional 6 days) or continuously radiated with red light for 21 days, as previously proved to be effective. Control (untreated) tomatoes were kept in the dark for the same period. Color parameters of the outer part were strongly influenced by the application of continuous red light. In different parts (outer and inner) of fruit, significant differences between treatments were analyzed for major compounds such as lycopene, β-carotene, total phenolic and total flavonoid concentration. Fruit treated with continuous red light showed the highest concentration in all parameters.

   In summary, application of continuous red light has proved effective to enhance color and firmness of tomato fruits by stimulating accumulation of carotenoids and antioxidative compounds during post-harvest storage and accelerating full tomato ripening.

Einfluss von Zusatzbeleuchtung während der Nachernte auf Reife- und Qualitätsentwicklung von Tomaten

Das Hauptziel der vorliegenden Arbeit war, die optimale Expositionsdauer sowie die Effekte von rotem Licht und kurzen Perioden von UV-Bestrahlung auf die Nacherntequalität von grünen Tomaten während der Reife zu bestimmen. Dies lieferte zugleich maßgebliche Erkenntnisse für den grundsätzlichen Mechanismus von Sekundärmetaboliten in Tomaten nach der Ernte unter optimalen Lichtbedingungen. Dazu wurden in einer mit Rotlicht emittierenden Dioden sowie mit UV-Röhren ausgestatteten Klimakammer Experimente durchgeführt. Die Versuche an grünen stage-1 Tomaten beinhalten Untersuchungen über den Einfluss von Licht auf die Fruchtfarbe, den Normalized Difference Vegetation Index (NDVI), den Normalized Anthocyanin Index (NAI), das Simple Chlorophyll Fluorescence Ratio (SFR_R) sowie die Fruchtfestigkeit und den Gehalt an löslichen Feststoffen (TSS) nach der Ernte. Die Gesamtmenge der Phenole,sowie die Flavonoidkonzentration und die antioxidative Kapazität wurden mittels eines Spektrometers analysiert. Die in den einzelnen Kapiteln im Detail ausgeführten Ergebnisse lassen sich wie folgt zusammenfassen:

1. Grüne Tomaten wurden 20 Tage lang täglich 30 Minuten lang UV-Strahlung, rotem Licht oder einer Kombination aus beidem ausgesetzt. Unbehandelte (Kontroll-)Früchte reiften binnen 15 Tagen, während Früchte, die rotem Licht oder einer Kombination aus rotem und UV-Licht ausgesetzt waren, das gleiche Reifelevel fünf Tage früher erreichten. Darüber hinaus erhöhten die alleinige Bestrahlung mit Rotlicht und die Bestrahlung mit einer Kombination aus Rotlicht und UV-Licht die Gehalte an Lykopin, ß-Karotin, Flavonoiden und Phenolen. Diese Möglichkeit der Steuerung der Konzentrationen von gesundheitsfördernden Antioxidantien ist eine verlässliche Methode zur Steigerung der Fruchtqualität im Sinne der Ansprüche und Bedürfnisse des Verbrauchers.
2. Der Einfluss der Einstrahlung von rotem Licht mittels LED-Modulen auf den Gehalt an gesundheitsfördernden Bestandteilen wurden an grünen stage-1 Tomaten ermittelt, die unterbrochener oder kontinuierlicher Bestrahlung ausgesetzt waren. Das erste Experiment erfasste die Gesamteffekte unterschiedlich langer Rotlicht-Einstrahlung (Dunkelheit, durchgängige Rotlicht-Einstrahlung für 10, 15 und 20 Tage), wohingegen das zweite Experiment den Fokus auf die Effekte unterbrochener Rotlichtbestrahlung legte (Dunkelheit, Rotlicht für 30 Minuten pro Tag, Rotlicht für 6 Stunden pro Tag, Rotlicht für 12 Stunden pro Tag, durchgängiges rotes Licht). In beiden Experimenten dienten dunkel gelagerte Tomaten als Kontrolle. Ununterbrochene Bestrahlung mit Rotlicht beschleunigte die Reifung grüner Tomaten. Darüber hinaus erhöhte dauerhaftes Rotlicht signifikant die Gehalte an Lykopin, ß-Karotin, Flavonoiden und Phenolen sowie die antioxidative Kapazität im Vergleich zu allen anderen Behandlungen. Dies lässt vermuten, dass durchgängige Rotlichtbestrahlung metabolische Prozesse positiv beeinflusst und zu höheren Gehalten gesundheitsfördernder Substanzen in Tomaten beiträgt.
3. Basierend auf diesen Erkenntnissen wurden nachfolgend grüne stage-1 Tomaten nach der Ernte entweder fünfzehn Tage lang täglich 12 Stunden mit Rotlicht bestrahlt (und anschließend 6 Tage dunkel gelagert), oder durchgängig 21 Tage lang mit rotem Licht bestrahlt, wobei unbehandelte Früchte dunkel gelagert wurden. Farbparameter der äußeren Gewebe der Frucht wurden durch die kontinuierliche Applikation von rotem Licht stark beeinflusst. Für unterschiedliche Bereiche der Frucht (innen und außen) wurden signifikante Unterschiede bei wichtigen Komponenten wie Lykopin, ß-Karotin, Flavonoide und Phenole in Abhängigkeit von der Behandlung ermittelt. Früchte, die mit kontinuierlichem rotem Licht behandelt wurden, zeigten die höchsten Konzentrationen aller Parameter. Insgesamt haben die Ergebnisse gezeigt, dass kontinuierliches Rotlicht als Fruchtreife-beschleunigende Methode eingesetzt werden könnte ohne sich dabei nachteilig auf den Gesamtgehalt gesundheitsfördernder Inhaltsstoffe auszuwirken.

   Abschließend lässt sich feststellen, dass eine kontinuierliche Applikation roten Lichtes sich als effektive Nacherntemaßnahme zur Verbesserung von Farbe und Festigkeit von Tomatenfrüchten erwies mit Auswirkungen auf die Akkumulation von Carotinoiden und antioxidativen Verbindungen sowie einer Beschleunigung der vollständigen Fruchtreife.

Contents

List of abbreviations

%	percent
℃	degree Celsius
a*	red to green
µg	microgram
µL	microliter
µm	micrometer
ABTS+	2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid)
am	ante meridiem
ANOVA	analysis of variance
b*	blue to yellow
CCD	carotenoid cleavage dioxygenases
CrtISO	carotene isomerase
d	day
DMAP	dimethylallyl diphosphate
DMRT	Duncan's Multiple Range Test
DXS	1-deoxy-d-xylulose
et al.	et alii (m.), et aliae (f.), and others
FRF_R	far-red fluorescence
g	gram
G3P	glyceraldehyde 3-phosphate
GAE	gallic acid equivalents
GGPP	geranylgeranyl diphosphate
GGPPS	geranylgeranyl pyrophosphate synthase
HAA	hydrophilic antioxidant activity
HPLC	high performance liquid chromatography
IPP	isopentenyl diphosphate
kg	kilogram
L	liter
L*	lightness
LAA	lipophilic antioxidant activity
LED	light emitting diode
LCY-B	lycopene β-cyclase
LCY-E	lycopene ε-cyclase
m	meter

mg	milligram
min	minute
mL	millilitre
mm	milimeter
mM	millimolar
n	number of replications
n.s.	non significant
NAI	the Normalised Anthocyanin Index
NCED	9-cis-expoxycarotenoid dioxygenases
NDVI	the Normalised Difference Vegetation Index
nM	nanomolar
nm	nanometer
PAR	Photosynthetic Active Radiation
PDS	phytoene desaturase
pm	post meridiem
PSY1	phytoene synthase
QAE	quercetin equivalents
RF_R	red fluorescence
s	second
SE	standard error
SFR_R	Simple Fluorescence Ratio
TE	Trolox equivalents
TSS	Total soluble solids
USDA	The United States Department of Agriculture
UV	ultraviolet
UV-A_{BE}	the biologically effective UV-A radiation
UV-B_{BE}	the biologically effective UV-B radiation
UV-C_{BE}	the biologically effective UV-C radiation
UV/Vis	ultraviolet–visible
v/v	volume by volume
VIS	visible
W	watt
ZDS	ζ-carotene desaturase
ZISO	ζ-carotene isomerase

A Introduction

1. Tomato as cultivated plant

Tomato belongs to the *Solanaceae* (nightshade family), genus *Solanum*, section *Lycopersicon* (Costa and Heuvelink, 2018) is grown for its edible fruit. Tomato fruit varies considerably in both their physical and chemical characteristics, and the common fruits form consumed today are quite different from those consumed just a few decades ago.

1.1 Importance of tomato fruit

Tomato is one of the world's major fresh and processed fruit and is the second most important vegetable crop after the potato (Costa and Heuvelink, 2018; Toor and Savage, 2005), especially in western countries (Willcox et al., 2003). Global tomato production is currently around 170 million tons, with 75% for the fresh market and 25% for processing (Costa and Heuvelink, 2018). Together with its derived products, tomatoes are one of the major food sources of carotenoids, providing an estimated 80% of daily intake of lycopene, in addition to folate, ascorbic acid, flavonoids, a-tocopherol and potassium in the western diet (Azari et al., 2010; Bramley, 2000; Khachik et al., 2002; Willcox et al., 2003). Several epidemiological studies have underlined the beneficial effect of tomato consumption in the prevention of chronic diseases such as cancer and cardiovascular disease (Klipstein-Grobush et al., 2000; Giovannucci et al., 2002).

1.2 Fruit quality

During the post-harvest chain (from harvesting to retailing) the concept of fruit quality is frequently used but its significance is different depending on the level at which it is used: growers, producers, handlers, packers, distributors, retailers, markets, and finally and the most important customers (Valero and Serrano, 2010). Quality of fresh tomato is a complex trait including multiple variables. Tomato color is the first external characteristic which determines the degree of consumer acceptance (Bertin, 2018). While

the commercial quality relies mainly on external attractiveness (such as color, shape, size, firmness and shelf-life), the organoleptic quality depends on physical (texture or firmness) and biochemical traits determining the overall taste and flavor (Batu, 1998). On the other hand, the health benefits rely on the consumption in vitamins and antioxidant compounds (lycopene, β-carotene, ascorbic acid and polyphenol) as well as minerals (potassium, calcium, phosphorus, potassium) (Bertin, 2018).

1.3 Nutritional value and health benefits

Nutritional components of tomato such as lycopene, phenolics, flavonoids and vitamins C and E are mainly responsible for the antioxidant activity of raw tomatoes and processed tomato products (Leonardi et al., 2000; Stewart et al., 2000). Meanwhile, fresh fruits have been recognized as a major source of vitamins and antioxidants and as important part of human diet and welfare on account of their nutritional value (Bertin, 2018). Results from epidemiological studies have shown that high consumption of tomato is consistently correlated with a reduced risk of some types of cancer (Franceschi et al., 1994) and may account for a low incidence of ischemic heart disease (Gerster, 1997). In tomatoes, the changes in content, chemical composition, and antioxidative properties during ripening depend on environmental factors such as temperature, light, water availability and nutrient availability (Cano et al., 2003; Jimenez et al., 2002), the agricultural techniques, cultivars, plant growth regulators and ripening stage (Kotíková et al., 2011; Ozgen et al., 2012). The red pigment in the tomato fruit, lycopene, is an antioxidant whose content in tomato fruit increases as the fruit ripens (Lopez et al., 2007). Thus, several attempts to produce a tomato with higher carotenoid content have been made.

1.4 Ripening behavior of tomato

Fruit ripening is a complex and highly coordinated developmental process that yields succulent and flavorful tissues for organisms that consume and disperse the associated seeds (Giovannoni, 2001). Tomatoes are climacteric fruits, which means that ripening can proceed after harvest. This process is characterized by the onset of a

climacteric rise in respiration, i.e., softening and the first appearance of red color (Lelievre et al., 1997). Ripening, or fruit maturation, is the physiological process giving rise to red, fully developed mature fruit. Therefore, during ripening, the fruit can be partially green and red. Under proper conditions of temperature and humidity, fruits progress through six well-defined stages to the red-ripe stage (Fig 1). These stages are: (1) Mature-green, (2) breaker, (3) turning, (4) pink, (5) light-red and finally (6) red-ripe; and they are based almost entirely on the external color change of the fruit from green to red (USDA, 1991).

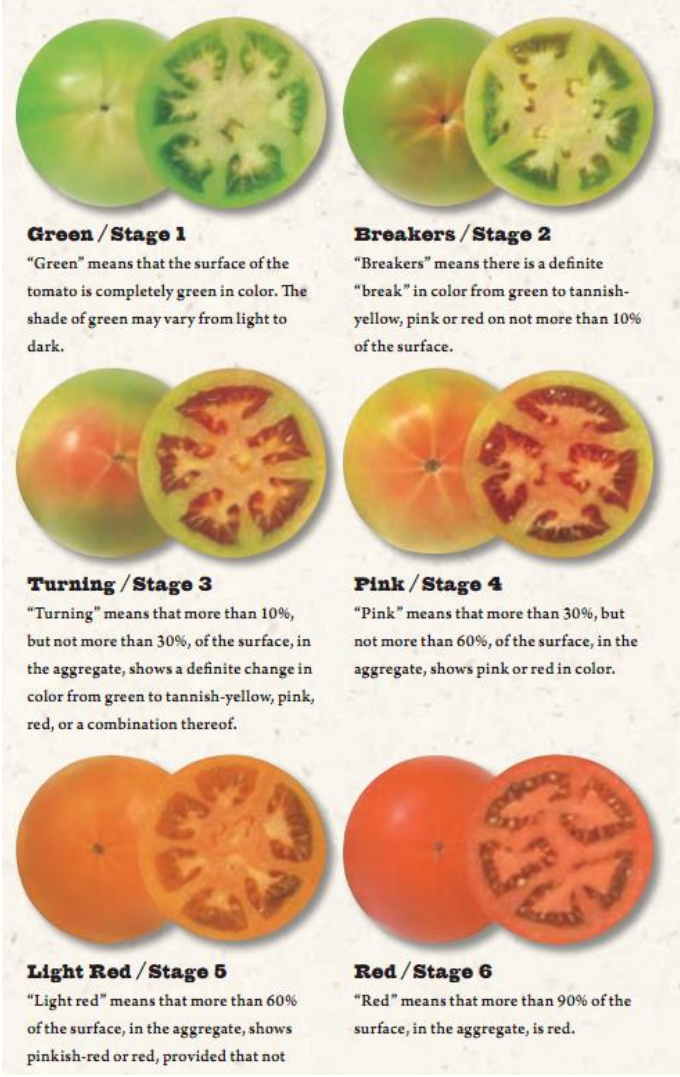

Figure 1. Classification of fresh market tomatoes based on changes in external and internal color. (source: USDA, 1991).

The actual time from anthesis until full maturity can vary tremendously among species/cultivars due to genetic and environmental differences. Even between fruit on the same plant, fruit development, and ripening can take more or less time depending on local microclimate conditions and differences in sink/source relations within the plant (Van de Poel et al., 2012). Proper harvesting determines the nutrient contents as well as storage ability of tomatoes. All over the world, tomatoes are harvested at different maturity stages, such as green immature stage, half ripen stage and red ripen stage. In the process of ripening, chlorophyll is degraded, and yellow orange carotenoid and red lycopene are synthesized.

Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes of color, texture, flavor, and chemical compositions. Ripening of tomato has been widely studied with the main objective to extend tomato consistency, color and shelf life. It has been shown that ripening processes and storage temperature can severely affect the final nutrient composition of the fruit (Madhavi and Salunkhe, 1998). However, high quality fruits should have uniform red color distributed over the entire surface of the fruit. For fresh tomatoes, color is the most important quality attribute, which directly relates to their marketing value (Tijskens and Evelo, 1994). Depending on consumer and market requirements, tomatoes are harvested at different stages of ripening from breaking to red color (Wold et al., 2004). During ripening, important biochemical processes occur. Some are beneficial for quality development such as development of color, accumulation of sugars and volatile compounds. Others are detrimental to long storage, such as loosening of the cell wall, which leads to loss of fruit firmness and reduction of shelf-life. In the climacteric fruit of tomato, the onset of ripening is preceded by the increase of respiration and the biosynthesis of ethylene (Javanmardi and Kubota, 2006).

2. Bioactive compounds and antioxidant activity during tomato fruit ripening

The ripening of tomatoes involves various morphological, physiological, biochemical and molecular changes including chlorophyll degradation and synthesis and storage of carotenoids, particularly lycopene, when chloroplasts are transformed to chromoplasts (Asada, 1994; Brandt et al., 2006; Lopez-Juez, 2007). The color transition

has been quantified based on color measurements expressed in the L*a*b*- color space (Hertog et al., 2004; Pinheiro et al., 2013) or in the RGB color space (Lana et al., 2006; Schouten et al., 2007). Most approaches are very similar and describe tomato color development as a process where a green pigment complex is converted into a red pigment complex resulting in a description of the color transformation as a logistic curve. Recently, Farneti et al. (2012) showed that the Normalised Anthocyanin Index (NAI) obtained from remittance VIS spectroscopy is closely related to the lycopene level in pericarp tissue as measured by HPLC. Another index, the Normalised Difference Vegetation Index (NDVI) is linked to the chlorophyll content (Zude, 2003). Calculating NAI and NDVI values from a tomato remittance VIS spectrum is expected to assess the level of lycopene and chlorophyll simultaneously.

Repeated non-destructive assessment over time of lycopene and chlorophyll levels of individual tomatoes allow for building a physiologically more correct kinetic model describing the transition from chloro- to chromoplast. Moreover, the multiparametric fluorescence sensor (commercial name Multiplex®, FORCE-A, Orsay, France) used in this study records the fluorescence signal emitted by chlorophyll molecules after excitation with radiation of defined wavelengths. The parameter Simple Fluorescence Ratio (SFR_R) is directly related to the chlorophyll concentration of the sample and considers the reabsorption of red light by the chlorophyll molecules (Groher et al., 2018).

Generally, the recommended harvest time for typical red tomatoes is at the mature green, breaker or pink stages, as this favors a long shelf life and is associated with optimal fruit firmness. Whilst, the fruit colors of green, yellow/orange, and red are generally attributed to chlorophyll, lutein/ β-carotene, and lycopene, respectively (Giovannoni, 2001; Heuvelink, 2005). However, the levels of lycopene, phenolic compounds and antioxidant activity are significantly influenced in a variety and maturity stage dependent manner (Martınez-Valverde et al., 2002).

2.1 Carotenoid compounds

Carotenoids comprise one of the largest classes of pigments in nature and grouping of lipid-soluble nature pigments present in fruit and vegetables that impart colors from yellow to red (Pecker et al.,1996). The green pigment chlorophyll degrades

and carotenoids are synthesized. Carotenoids, particularly lycopene and β-carotene, represent the primary components of ripe fruit pigmentation in tomato pericarp and are responsible for the characteristic color of ripe tomatoes, conferring deep red and orange colors, respectively (Tijskens and Evelo, 1994). Carotenoid formation during tomato fruit ripening has been studied extensively and has become the best model system for the other chromoplast-containing tissues. During ripening, the concentration of carotenoids increases between 10- and 14-fold, due mainly to the accumulation of lycopene (Fraser et al., 1994). The major carotenoids that accumulate in ripe red fruits are lycopene (~90%), β-carotene (5–10%), and lutein (1–5%), with trace amounts (<1%) of other carotenoids (Ronen et al., 1999).

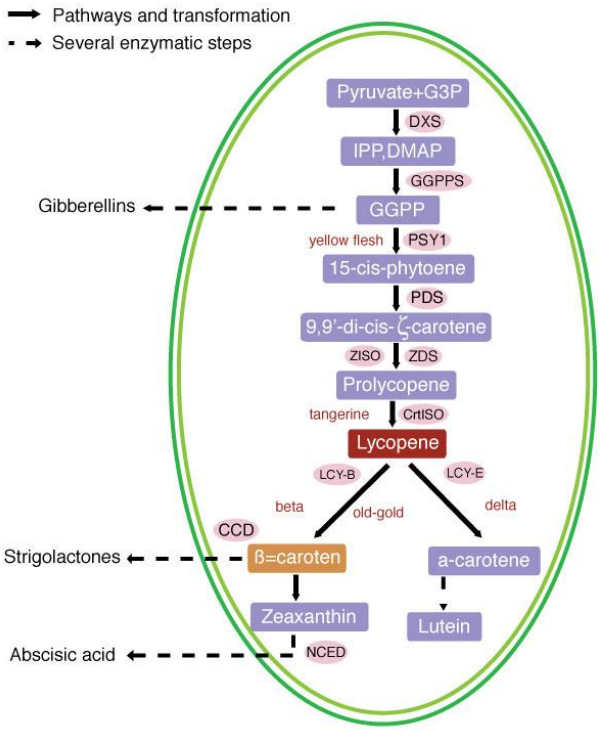

Figure 2. Engineering of carotenoid biosynthesis and catabolism (slightly modified after Lui et al., 2015).

During the chloroplast to chromoplast transition, specific carotenoid biosynthesis genes are expressed. The first committed step in carotenoid biosynthesis corresponds to the condensation of two geranylgeranyl diphosphate (GGPP) molecules into phytoene

(Bergougnoux, 2014), which is catalyzed by phytoene synthesis (PHY). In tomato fruit, two PHY genes are expressed. Phytoene synthesis 1 (PSY1) is highly expressed in ripening fruit and is responsible for the formation of chromoplastic carotenoids, whilst phytoene synthesis 2 (PSY2), which is responsible for the formation of chloroplast carotenoids, is expressed exclusively in green tissue and therefore makes no contribution to carotenoid biosynthesis in ripening fruit (Fraser et al., 1999; Giorio et al., 2008). The accumulation of lycopene in regular tomatoes is related to the low expression of the lycopene β-cyclase (LCY-B) gene involved in the conversion of lycopene into β-carotene (Fig. 2) (Ronen et al., 1999). Proteomics studies dealing with the quantitative analysis of proteins involved in the carotenoid pathway during the transition from mature green to red tomatoes have shown that PHY1, ζ-carotene desaturase (ZDS) and carotene isomerase (CrtISO) undergo a strong increase in abundance, whilst geranyl desaturase remains equally abundant during the chloroplast to chromoplast transition. Interestingly, proteins downstream of lycopene, such as LCY-B were detected at low levels only and could not be quantified. When tomato fruit accumulate β-carotene, the expression of the LCY-B gene is elevated (Ronen et al., 1999), thus demonstrating the crucial role of LCY-B in controlling the accumulation of lycopene or β-carotene.

Lycopene is a powerful natural antioxidant that acts as the most efficient singlet oxygen quencher in vitro among common carotenoids, and β-carotene is the precursor of vitamin A, which has a high antioxidant property, making it of interest in human health (Mascio et al., 1989).

2.2 Polyphenols

Polyphenols represent a large class of plant secondary metabolites, while many recent papers refer to all phenolics compounds as polyphenols (Quideau et al., 2011). Phenolics as a group represent the strongest antioxidants in fruits and vegetables, although the antioxidant activity of individual phenolic compounds may vary depending on their chemical structure (Scalzo et al. 2005; Valero and Serrano, 2010). The most common phenolics in human diet are phenolic acids, flavonoids and tannins (King and Young, 1999). Tomatoes represent the predominant source of antioxidants, and besides the carotenoids, the flavonoids have been confirmed as a group of polyphenols important,

up to 200–300 mg of fresh weight, in conferring antioxidant benefits (Luthria et al. 2006; Scalbert, 2005; Slimestad and Verheul 2005). In recent years, several studies have already looked at the influence of genotypes (George et al. 2004) and levels of fruit maturity (Buta and Spaulding 1997), as well as agronomical practices, on the content of phenolic compounds in tomatoes (Dumas et al. 2003).

Macheix et al. (1990) showed that, in addition to genetic control, which is the main factor in determining phenolic compound accumulation in vegetable foods, external factors may also have a significant effect. In cherry tomatoes, the increase in phenolic content is ascribed to an increased solar radiation received by fruits (Wilkens et al. 1996; Raffo et al. 2006). Brandt et al. (1995) mentioned that the flavonol content in some plant species may be enhanced by the exposure of the plants to increased UV-B radiation. On the other hand, only limited or no data was found in the literature dealing with light irradiation as another important environmental factor influencing the content of phenolic compounds (Dumas et al. 2003).

2.3 Antioxidant activity

The role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices, post-harvest storage, processing and ripening stage of the fruit (Cano et al., 2003; Dumas et al., 2003; Binoy et al., 2004; Garcia and Barrett, 2006; Liu et al., 2011). In addition, the qualitative and quantitative analysis of different antioxidants, as well as their variation during ripening, is of great relevance both to human health and to commercial purposes (Ilahy et al., 2011; Jimenez et al., 2002; Zobel, 1997). It is well-known that tomato ripening involves a number of physiological processes that include the visible breakdown of chlorophyll and build-up of carotenoids, with massive accumulation of antioxidant components such as lycopene and β-carotene (Laval-Martin et al., 1975) within the plastids. Besides carotenoids, the contents of other important antioxidant compounds, such as ascorbic acid, α-tocopherol and phenolics, should be highlighted thus varying the nutritional value and the antioxidant activity of the tomato (Cano et al., 2003; Giovanelli et al., 1999; Martinez-Valverde et al., 2002; Raffo et al.,

2002; Slimestad and Verheul, 2005). Moreover, ripening conditions affect both the antioxidant accumulation kinetics and the final contents, which are higher in post-harvest ripened than in vine ripened fruit (Giovanelli et al., 1999).

3. Secondary metabolites in tomato fruit tissues during ripening

The tomato fruit is composed of several different tissues and cell layers (Fig. 3). Tomato fruit skin (or peel) and flesh, these tissues vary in terms of transcript and metabolic profiles (Mintz-Oron et al., 2008; Moco et al., 2007). Mintz-Oron et al. (2008) described a comparative transcriptome and metabolome analysis of skin and flesh tissues during five stages of tomato fruit development. Metabolite profiling revealed 100 metabolites that were enriched in the skin tissue during development and 45 secondary metabolites were identified that were at least two-fold up-regulated in the skin compared with the flesh tissue.

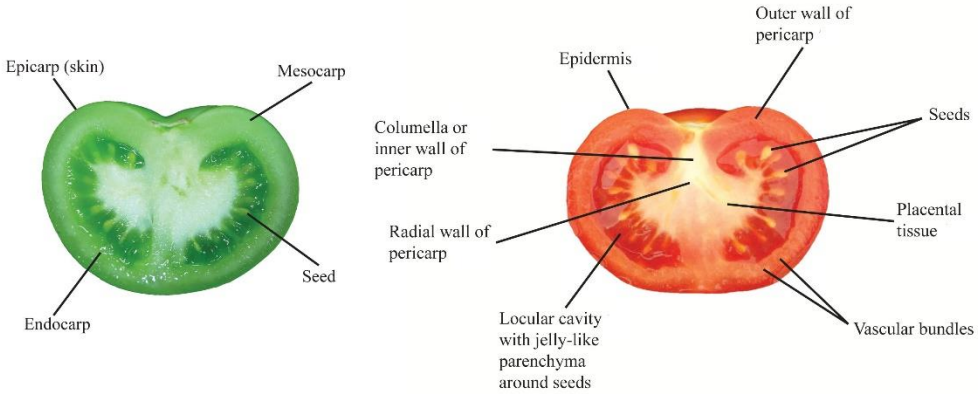

Figure 3. Anatomy of tomatoes (Photo: Panjai, 2017).

Several studies have already shown that the skin of some tomato fruits contains significantly higher levels of lycopene, phenolics, flavonoids, ascorbic acid and antioxidant activity than pulp and seed fractions (Reboul et al., 2005; Shi and Le Maguer, 2000 Toor and Savage, 2005). Tomato skin can contain about five times more lycopene (540 mg kg^{-1}fw) than tomato pulp (110 mg kg^{-1}fw) (Dumas et al., 2003). Due to, the skin prevents direct incidence of light on the pulp, another factor that has been linked to the deterioration of bioactive compounds (Lee and Chen, 2002; Peng et al., 2008).

Furthermore, Sharma and Le Maguer (1996) observed that most of the lycopene was associated with the skin and water-insoluble fraction of the tomato pulp. Similarly, George et al. (2004) studied antioxidant compounds in 12 field-grown tomato genotypes and reported that on average, the tomato skin had 2.5 times higher lycopene levels than the pulp. In tomato fruit, the total phenols, present in the epidermal tissue, the placental tissue, the radial and inner walls of the pericarp and the outer wall of the pericarp, did not vary significantly among the three cultivars tested Patriot, Floridade and Walter (Senter et al., 1988).

Senter et al. (1988) found that the levels of the total phenols measured in the epidermal tissue, in the placental tissue, in the radial and inner wall of the pericarp and in the outer wall of the pericarp were 30.2 g kg^{-1}, 25.2 g kg^{-1}, 20.8 g kg^{-1} and 19.4 g kg^{-1} dry tomato tissue, respectively. Moreover, Stewart et al. (2000) reported that the majority of flavonols in tomatoes are present in the skin. Phenolic compounds in tomato fruit are concerned, 98% of flavonols detected, primarily as conjugates (quercetin and kaempferol), were found to occur in the skin. The total flavonol content can strongly vary, for example as shown for 20 fresh tomato varieties revealing a range from 1.3 to 22.2 mg kg^{-1} fresh weight (Stewart et al., 2000).

4. Effects of irradiation on tomato fruit ripening

Light is one of the most important environmental factors affecting the pigment metabolism of vegetables and fruits (Lado et al., 2015; Zhan et al.,2012). The role of visible light in food production, as in agriculture and horticulture, is obvious, as light drives photosynthesis, which is crucial for plant growth and development. Moreover, light stimulates the biosynthesis of plastid structures to accommodate these photoprotective pigments (Llorente et al., 2017). It is now understood that low quantities of light can maintain the post-harvest quality of crops by mitigating senescence and improving phytochemical and nutrient content in several species (Braidot et al., 2014; Costa et al., 2013; Glowacz et al., 2014; Pogson and Morris 2004). Whilst, light-emitting diodes (LEDs) are solid-state lighting devices that emit light with emission wavelengths of narrow bandwidths, high photoelectric efficiency and photon flux or irradiance, low thermal output, compactness, portability, and which are easily integrated into electronic

systems (Branas et al., 2013). The properties of LED that are useful in horticultural production include the ability to control the quality of light, the limited amount of heat generated, as well as the ease of integration into electronic systems to give greater control over the emitted light. Light plays a role in the evolution of the color of tomatoes after harvest as it affects the metabolism of pathways involved in biosynthesis of pigments (Azari et al., 2010; Li et al., 2013; Toledo-Ortiz et al., 2010). Additionally, at favorable temperatures (22–25 ◦C), the rates of synthesis of lycopene and carotene can be increased by illuminating tomato plants during the ripening of the fruit. Fruit exposed to direct sunlight during its development had higher carotene levels than shaded fruit (McCollum, 1954). Particularities of the light spectrum affect the pigments synthesized, which play a decisive role on the shelf lives of tomatoes. Although the impact of light and LED on horticultural plant development and metabolism under different light combinations was highlighted for some plant species (Bantis, et al., 2018), studies on the simultaneous effect of LED on the content of secondary metabolites in fruits are still missing.

4.1 Effects of UV light on fruit

The sterilizing capabilities of ultraviolet (UV) radiation (100–400 nm) are well known, yet invisible light has been shown to have bactericidal effects under certain conditions, hence playing a role in food safety (D'Souza et al., 2015). UV radiation can effectively penetrate into the plant tissues and be absorbed. Irradiation with UV light has been shown to extend freshness during storage and improve nutrients and quality of vegetables and fruit (Kim et al., 2011; Liu et al., 2009). For instance, UV light delays chlorophyll degradation, reduces tissue damage and disruption, and maintains antioxidant capacity, ultimately extending the storage period of broccoli (Costa et al., 2006). Maneerat, Hayata, Muto and Kuroyanagi (2003) reported that UV-A irradiated tomatoes show normal color development and fruit ripening without any physiological disorder. Although there is more research going on the effect of post-harvest UV-B treatment on other crops, such as broccoli (Aiamla-or et al., 2009; 2010), grapes (Cantos et al., 2000), mushrooms (Roberts et al., 2008), apples (Hagen et al., 2007), nasturtium (Schreiner et al., 2009), and enhanced level of antioxidant compounds and antioxidant enzyme activity

in plants (Costa et al., 2002; Xu et al., 2008), very limited study has been done in case of tomato fruit in the past decades.

Barka et al. (2000) also reported that treatment of green tomatoes with UV-C light (peak output of 254 nm) reduces activity of cell-wall degrading enzymes. Additional studies have reported that low-dose UV-C can induce resistance to Rhizopus soft rot, delay ripening, improve firmness and extend the shelf-life of tomatoes (Liu et al., 1993; Stevens et al., 2004). Bu et al. (2013) previously reported that UV-C maintained the firmness of Cherry tomatoes (*Solanum lycopersicum* L. cv. Zhenzhu1.), with decreased expression of cell wall degrading enzymes. In comparison, Tiecher et al. (2013) observed delay in fruit maturation without a commensurate prolongation of tomato firmness (*S. lycopersicum* cv. Flavortop). Obande et al. (2011) reported on pre-harvest UV-C treatment of tomatoes (*S. lycopersicum* L. cv. Mill.) and influences on the firmness of fruit with varying results depending on the applied dose. Many studies have been conducted on the effects of UV-C irradiation on delaying the loss of firmness and changes in composition and structure of the cell wall. However, little information is available on the mechanisms of UV-C irradiation in conserving fruit firmness.

However, investigations on the effects of UV-C treatments, such as post-harvest UV irradiation, on the ripening of fresh tomatoes have resulted in different conclusions regarding the appropriate radiation intensity, the optimal ripening stage of tomatoes, and the storage conditions. For example, Liu et al. (1993) found that the prolonged exposure of tomatoes to UV-C accelerates their ripening and senescence, but higher doses impaired ripening and caused abnormal browning, manifesting as sun scalding on the fruit surface (Maharaj et al., 1999). Whilst et al. (1999) have reported that UV-C irradiation at 3.7 kJ m^{-2} and 24.4 kJ m^{-2} delays the development of tomato tissue color and softening.

Exposure to UV light causes stress in plant tissues, which stimulates the biosynthesis of defensive secondary metabolites with antioxidant activity; these are mainly lycopene in tomatoes (Liu et al., 2009) and phenolic compounds in grapes and tomatoes (Cantos et al., 2000; González-Barrio et al., 2009; Jagadeesh et al., 2009; Liu et al., 2009). Due to UV-C irradiation, ascorbic acid, lycopene and total phenolic contents increased in tomato fruit (Jagadeesh et al. 2009). However, a dose higher than the hormetic dose is found to impair ripening and cause abnormal browning in tomato fruit (Maharaj et al. 1999). Recently, a number of specific post-harvest elicitor treatments, such

as low or high temperature treatments, ultraviolet and gamma irradiation, altered gas composition, may further enhance antioxidant activity (Lui et al., 2012).

A study by Liu et al. (2009) has reported an increase in the lycopene content of UV-C-treated tomato fruit. UV-C radiation has been shown to reduce pathogen inoculum and promote accumulation of specialized metabolites (Maharaj et al., 1999; Charles et al., 2008a,b). To exemplify, UV-C treatment induced synthesis of phenolic compounds in tomato (Bravo et al., 2012; Jagadeesh et al., 2009). Barka (2001) observed increased activity of antioxidant enzymes in tomatoes exposed to UV-C radiation. Moreover, Liu et al. (2009) and Bravo et al. (2012) found higher lycopene contents in tomatoes treated with UV-C, and Stevens et al. (1998) observed higher polyamine contents in UV-C treated tomatoes. Post-harvest UV-C treatment of fruit and vegetables, therefore, has the potential to become a technological alternative to improve conservation. The application of hormetic doses of UV-C cannot only improve storage potential, but also increase nutritional and functional properties of fruit and vegetables.

4.2 Effects of red light on fruit

Red light (635-700 nm) is important for the development of the photosynthetic apparatus and increases flowering, budding and starch accumulation in plants (Saebo et al., 1995; Wu et al.,2007). Early studies indicated that phytochromes mediate light-induced carotenoid biosynthesis in tomato by conducting red and far-red light during ripening (Khudairi and Arboleda, 1971; Thomas and Jen, 1975). Alba and Cordonnier-Pratt (2000) reported that red light treatments (six 40 W Gro-lux lamps) increased lycopene accumulation 2.3-fold in tomatoes and that red light-induced lycopene accumulation was reversible by far-red light treatment. They concluded that the accumulation of lycopene was under the control of fruit-localised phytochromes. They hypothesize that phytochrome might be involved (Dumas et al., 2003).

Phytochromes are photo-reversible pigments, that exist in two interconvertible forms, Pfr absorption maximum 730 nm and Pr absorption maximum 660 nm, but only red light can activate the protein to induce a physiological response (Fig. 4) (Borthwick et al., 1952; Borthwick, 1972; Quail, 2002). Phytochromes have been implicated in regulating the extent of lycopene accumulation in tomato fruit (Alba et al., 2000). When

incubated in darkness interrupted by red light, or red light followed by far-red light, pericarp discs from breaker stage fruits accumulated higher levels of carotenoids than darkness and red light/far-red light-treated discs (Schofield and Paliyath, 2005).

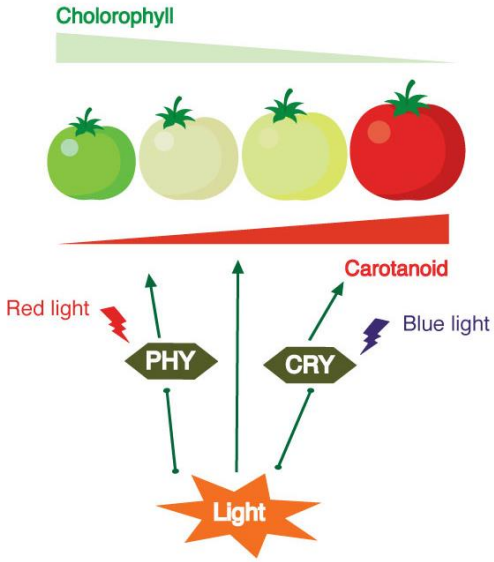

Figure 4. The elegant modulation of carotenoid synthesis by red light in tomatoes (slightly modified after Lui et al., 2015).

In tomatoes, the accumulation of lycopene along with an increase in total carotenoid content, was also observed in response to red light treatment (Alba et al., 2000; Schofield and Paliyath, 2005; Liu et al., 2009). Other studies have shown that red light treatment increases the carotenoid content and red color of tomatoes, with varying effects on tomato firmness (Lee et al., 1997; Liu et al., 2009). More recently, it has been shown that brief red-light treatment of harvested mature green fruit stimulated lycopene accumulation 2.3-fold during fruit development (86.6 mg kg^{-1} fresh weight at the red ripe stage), compared with a dark control treatment (37.2mg kg^{-1} fresh weight) (Alba, 2000). Moreover, in citrus fruit, red light was effective in enhancing carotenoid content, especially the content of carotenoid content, inter alia β-cryptoxanthin, in the flavedo of Satsuma mandarin (Ma et al., 2012a). However, the precise optimal red light irradiation on secondary metabolites in tomato fruit after harvest has not been exploited in recent literature, yet.

5. Objectives and hypotheses of the study

Many studies have been conducted on the effects of red light and UV irradiation on physiological and biochemical processes in response to different light irradiation in tomato plants and fruits. However, no study has examined the effect of red light and UV combination on the ripening time of tomatoes. Instead of, most of the research works describe tomato biochemical composition in the fully ripen fruits (in red tomato) at the technical fruit maturity stage. To better understand the synthesis of carotenoids and other biochemical compounds, their concentration should be compared not only in red fruits, at the last stage of maturity, but also during all fruit ripening period, from green tomato fruit ripening stage to the technical fruit maturity.

The first main objective of this thesis was to investigate the potential of continuous red light and short periods of UV radiation to shorten post-harvest ripening time and to increase concentrations of some secondary metabolites and antioxidant activity in green stage tomatoes during post-harvest storage. A second objective was to investigate the effect of post-harvest red light radiation schemes (intermittent or continuous) on health-promoting compounds in tomatoes. The third objective was to investigate the effect of red light irradiation during post-harvest on external fruit color, lycopene, β-carotene, total phenolic and total flavonoid concentrations in the outer and inner parts of tomatoes.

The present thesis is divided into three separated experimental chapters with the respective hypotheses, as follows:

1. Red light and UV induce an accelerated chlorophyll breakdown of green tomato fruits in the ripening process of tomato fruits.

2. Different red light irradiation times per day lead to a differentiated synthesis of health-promoting compounds in tomato fruits.

3. Red light radiation accelerates color development by directly linking skin color formation and lycopene synthesis in the outer and inner tissue of tomato fruits.

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B Effects of continuous red light and short daily UV exposure during post-harvest on carotenoid concentration and antioxidant capacity in stored tomatoes[1]

1. Introduction

Fruit ripening is a complex, genetically programmed process that comprises changes in color, texture, flavor, and chemical composition (Javanmardi and Kubota, 2006). Tomato is a climacteric fruit and continues to ripen after harvest. The United States Department of Agriculture (USDA) established a color classification system, which is widely used to differentiate the ripeness of tomatoes (USDA, 2005). The ripening stage of tomato fruit is usually defined on basis of external color, which changes due to the degradation of chlorophyll and the biosynthesis of lycopene, the most abundant carotenoid (López et al., 2007), as well as ß-carotene, a precursor of vitamin A (Hobson and Grierson, 1993).

The health promoting benefits of tomato and tomato products have mainly been attributed to the significant amount of natural antioxidants, especially lycopene (Ilić et al., 2012). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu et al., 1998). In the human body, the oxidative metabolism can induce continuous production of free radicals. These highly aggressive compounds can cause permanent cell damage, leading to mutation and possibly cancer in human cells (Choudhary and Walters, 2013).

Lycopene is presently commercialized as a potent antioxidant and fortified nutritional supplement (Kaur and Kapoor, 2008). Epidemiological studies have shown that the increased consumption of lycopene rich food is associated with lower risk of cancer (Giovannucci, 1999). Furthermore, results suggest that lycopene plays a role in the prevention of different health issues, such as chronic diseases, cardiovascular

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disorders, digestive tract tumors, and can also inhibit prostate carcinoma cell proliferation in humans (Levy and Sharoni, 2004). In tomatoes, the changes in content, chemical composition, with different light spectra (Alba et al., 2000; Liu et al., 2003; Rosati et al., 2000). Excessive exposure to UV light causes stress in plant tissues and stimulates the biosynthesis of defensive secondary metabolites with antioxidant and screening activity. Examples of these compounds include lycopene in tomatoes (Liu et al., 2009) and phenolic compounds in grapes and tomatoes (Cantos et al., 2000; González-Barrio et al., 2009; Jagadeesh et al., 2011; Liu et al., 2009). Moreover, UV-B irradiation is considered being a useful non-chemical way of maintaining post-harvest quality and enhancing antioxidant capacity of tomato fruit (Liu et al., 2011). As reported, UV-C exposure in low dose might delay ripening, improve firmness and extend the shelf life of tomatoes (Stevens et al., 2004). Red light treatments (5 min of red light with 15 min of far-red light) increased lycopene accumulation 2.3-fold in tomatoes (Alba et al., 2000), indicating that the accumulation of lycopene was controlled by fruit-localised phytochromes. Other studies have shown that red light treatment increases the carotenoid content and red color of tomatoes, with varying effects on tomato firmness (Lee et al., 1997; Liu et al., 2009). However, no study has examined the effect of red light or UV on the ripening time of tomato yet. In addition, it is so far unclear how different light treatments can influence antioxidant activity in tomato fruit. Therefore, the objective of this research was to investigate the potential of continuous red light and short periods of UV radiation to shorten post-harvest ripening time and to increase concentrations of lycopene, β-carotene, total flavonoid and phenolic content as well as hydrophilic and lipophilic antioxidant activity in green stage tomatoes during post-harvest storage.

2. Material and methods

2.1 Tomato cultivation

Tomato (*Solanum lycopersicum* L.) fruit of the cultivar Cappricia (RijkZwaan, De Lier, The Netherlands) were harvested from plants cultivated in a commercial-like greenhouse at the Campus Klein Altendorf research station (University of Bonn, Germany, 50°37′31.6″N 6°59′18.1″E, altitude 600 m). Fruit with calyx were harvested at

the green stage 1 of maturity (USDA, 2005), as detailed in section 2.3. In order to restrict potential influences of developmental and environmental factors, one healthy tomato fruit of pre-defined size was harvested from a truss from different plants (always from the same position). Afterwards, tomatoes were placed into plastic trays covered with aluminum foil (30 fruit per tray), ensuring that the fruit did not touch each other. The trays were stored in a custom-built climate chamber for 20 d under constant day/night temperatures (20 °C/19 °C) with variable, day/night temperature-dependent relative humidity (75%/85%).

2.2 Light treatments

For this experiment, four different treatments were used:

1) Darkness (control)
2) Darkness + UV
3) Red light
4) Red light + UV

In treatment 1 (control), tomato fruit were placed in a box and kept in the dark in the same climate chamber, but separated via a cardboard to shield them from the light. For treatment 2 and 4, tomato fruit were additionally irradiated with UV light for 15 min every day in the morning (at 6.15 am) and at night (at 7.30 pm) with UV tubes (UVXEFL 290BB, Ushio Lighting Inc., Japan). Tomato fruit were exposed to UV light of 4.98 kJ m^{-1} fo 30 min per day which is equivalent to a biologically effective UV radiation of 5.53 kJ m^{-1} 30 min per day (UVBBE = 4.5, UV-CBE = 1.0, UV-ABE = 0.03 per 30 min per day) (Hoffmann et al., 2015). For treatment 3 and 4, the tomato fruit were irradiated with special light emitting diode (LED) modules (Ushio Lighting Inc., Tokyo Japan) installed in the climate chamber. This prototype, optimized for our research purpose, consisted of the following spectrum: 60% UV-B (280–320 nm with a dominant peak at 290 nm), 30% UV-A (320–400 nm), 4% UV-C (200–280 nm) (Hoffmann et al., 2015). The LED settings (intensity and spectral composition) were controlled by the equipment specific-software. The red light was applied for the whole storage period (red light peak at 665 nm) which is equivalent to a Photosynthetic Available Radiation (PAR) of 113 µmol m^{-2} per day (X1-2 SN4962 M RS232 optometer, Gigahertz-Optik GmbH, Germany). All tomato fruit

were carefully turned over every day (at 2 pm) to ensure light exposition of both fruit sides.

2.3 Tomato fruit sampling and remittance determinations

Six tomatoes were sampled on harvesting day (day 0) to characterize the quality (stage) at the starting point. External fruit color was assessed visually according to the Standards for Grade of Fresh Tomatoes established by the United States Department of Agriculture (USDA, 2005). For rating, the following scale was used:

1 = green, 100% green

2 = breaker, a noticeable break in color with less than 10% of color other than green

3 = turning, between 10 and 30% reddish color

4 = pink, between 30 and 60% reddish color

5 = light red, between 60 and 90% red

6 = red, more than 90% red

2.4 Remittance analysis

A hand-held spectrophotometer (Pigment Analyzer 1101, Control in Applied Physiology GbR, Germany) was used for non-destructive remittance analyses, including the Normalised Difference Vegetation Index (NDVI, estimating the chlorophyll concentration) and the Normalised Anthocyanin Index (NAI). Three points on each fruit were evaluated every 5 d, and an average NDVI and NAI per tomato fruit was calculated the following way:

NDVI = (R780-R660)/(R780 + R660)

NAI = (R780-R570)/(R780 + R570)

2.5 Sample preparation for destructive analyses

After the initial harvest, sampling was performed every 5 d (Day 0, 5, 10, 15 and 20). For this purpose, six fruits were randomly chosen from each treatment. After analysis

of above-mentioned optical and sensor-based properties, fruit were cut into small pieces and kept at −80 °C for lyophilisation (Gamma 1-16LSC, Christ, Osterode am Harz, Germany). Dried samples were ground and stored in the dark at room temperature until further preparation, extraction procedures and lab analyses.

2.6 Total soluble solids (TSS)

TSS represents an index of soluble solids concentration in fruit. A single drop of juice from homogenate, ground tomato tissue was put on a digital refractometer (Pocket PAL-1, ATAGO, Tokyo, Japan). Results were expressed as%.

2.7 Extraction procedure

The methanolic extraction 80% methanol [PubChem CID: 887] + 1.0% hydrochloric acid ([PubChem CID: 313] [37%, Merck, Germany]) described previously (Ponmozhi et al., 2011) with slight modifications was used for hydrophilic antioxidant activity, total phenolic content and total flavonoid assay. Petroleum ether (60%) was used for lipophilic antioxidant activity, lycopene and β-carotene assay. Samples of 0.05 g freeze dried tomatoes was placed in 1.5 mL of Eppendorf tube and 1.0 mL of the respective solvent was added. The mixture was vortexed (Vortex ex-2 Gene, Scientific Industries, New York, USA) for 20 s and sonicated for 5 min in an ultrasonic bath (Sonorex Super RK106, Bandelin electronic, Berlin, Germany), then centrifuged at 15,682g for 6 min to separate the supernatant (Eppendorf centrifuge 5415R, Hamburg, Germany). This procedure was performed four times. The supernatant was adjusted to 5 mL with the solvent and filtrated through a 0.20 µm membrane (regenerated cellulose, Phenomenex, Torrance, California, USA).

2.8 Lycopene and β-carotene

Lycopene and β-carotene concentrations were determined by using high performance liquid chromatography (HPLC, Agilent 1260-series, Waldbronn, Germany) according to the method of Olives et al. (2006). For quantification, the HPLC was

equipped with a reverse-phase system with an auto sampler and a Eurospher100-C18 column (250 x 4 mm, 5 µm packing material, KNAUER Wissenschaftliche Geräte GmbH, Germany). The detector was set at 475 nm and the column (temperature 30 °C) eluted with a mobile phase containing methanol/ACN (PubChem CID: 6342) (90/10 v/v) with triethylamine (TEA) (PubChem CID: 8471) 9 µM. The results were calculated to milligram of lycopene and β-carotene per kilogram by Chemstation software. Lycopene (PubChem CID: 446925) and β-carotene (PubChem CID: 5280489) were used as the standard for a linear calibration curve between 0.02 and 20 µg mL^{-1} and the results were expressed as milligram per kilogram dry mass.

2.9 Total phenolics

Total phenolic content was measured by using the Folin-Ciocalteu method described previously (Chen et al., 2015) with slight modifications. Briefly, 0.2 mL of the methanolic extracted sample was adjusted to 0.5 mL with water. The mixture was added to 0.5 mL 1:10 diluted Folin-Ciocalteu reagent. After 4 min of incubation, 1.5 mL of saturated sodium carbonate (PubChem CID: 516892) solution (75 g L^{-1}) was added. After incubation at room temperature for 120 min, the absorbance of the mixture was measured at 760 nm by using a spectrophotometer (Lambda 35 UV/Vis spectrometer, PerkinElmer, Boston, Massachusetts, USA) and the respective solvent as blank. Gallic acid (PubChem CID: 370) was used as the standard for a linear calibration curve between 50 and 500 mg L^{-1} and the results were expressed as milligram of gallic acid equivalents (mg GAE g^{-1}) dry mass.

2.10 Total flavonoids

Total flavonoid content was analysed by using the colorimetric method described previously (Chen et al., 2015) with slight modifications. Briefly, 1 mL of methanolic extract was mixed with 0.1 mL of 5% sodium nitrite (PubChem CID: 23668193) solution. After 6 min of incubation, 0.1 mL of 10% aluminum chloride (PubChem CID: 24012) solution was added and the mixture was allowed to react for another 5 min before adding 0.1 mL of 1 M sodium hydroxide (PubChem CID: 14798). The mixture was vortexed for

10 s and was then recovered with 1.7 mL of water. After 30 min of incubation, the absorbance was measured at 510 nm using a spectrophotometer (Lambda 35 UV/Vis spectrometer, PerkinElmer, Boston, Massachusetts, USA) and the respective solvent as blank. Quercetin (PubChem CID: 5280804) was used as the standard for a linear standard curve between 100 and 1000 mg L^{-1} and the results were expressed as milligram of quercetin equivalents (mg QAE g^{-1}) dry mass.

2.11 Hydrophilic and lipophilic antioxidant activity

The hydrophilic and lipophilic antioxidant activities (HAA and LAA) were examined by the 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS+, PubChem CID: 5360881) decolorisation method as described by Thaipong et al. (2006) with minor modifications. The stock solution was prepared by mixing 8 mL of 7 nMABTS solution (0.0384 g of ABTS+ adjusted to 10 mL of water) and 12 mL of 2.45 nM potassium persulfate (PubChem CID: 24412) solution (0.0166 g of K2S2O8 adjusted to 25 mL of water) and allowing them to react for 12 h at room temperature in the dark. The fresh solution was then diluted by mixing the ABTS solution with methanol to obtain an absorbance of 0.8–1.0 units at 734 nm by using the spectrophotometer (Lambda 35 UV/Vis spectrometer, PerkinElmer, Boston, Massachusetts, USA). The extractions (80 µL) were allowed to react with 1960 µL of the ABTS solution for 2 h in dark conditions. Then, the absorbance was taken at the same wavelength. Trolox (PubChem CID: 6541354) was used as the standard for a linear standard curve, which was generated between 50 and 500 µM Trolox mL^{-1}. The results were expressed in mM Trolox equivalents (TE g^{-1}) dry mass.

2.12 Statistical analysis

The results are expressed as means ± SE. Significant differences between treatment samples for all parameters were determined using a one-way ANOVA. A Duncan's Multiple Range Test was conducted to establish the differences among mean values. Statistical analyses were carried out using SPSS 22.0. The threshold p-value chosen for statistical significance was $p \leq 0.05$.

3. Results

3.1 Remittance analysis

The NDVI, in our study used as reliable indicator of chlorophyll breakdown, significantly decreased after 10 d in fruit treated with red light and red light with UV (Fig. 1A, Table 1). Control fruit and those radiated with UV only showed a significant decrease in NDVI after 15 d of exposure. The NAI significantly increased after 10 d of harvesting for fruit treated with red light and red light with UV (Fig. 1B, Table 1).

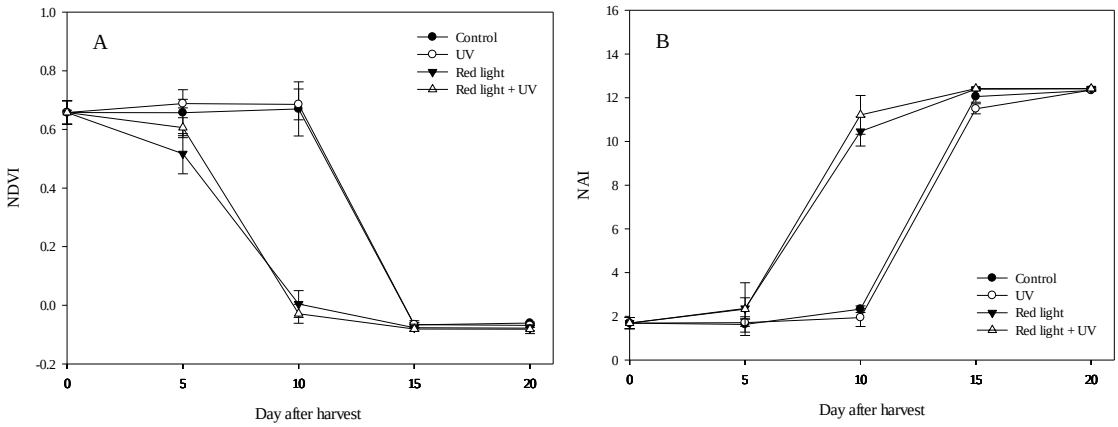

Figure 1. (A) NDVI and (B) NAI of control (closed circle) tomato fruit, fruit treated with UV (open circle), red light (closed triangle) and red light with UV (open triangle) on harvest day (0) 5, 10, 15 and 20 d after harvesting. Means ± SE, n = 6.

Table 1. NDVI, NAI and total soluble solids (TSS) of control tomato fruit, fruit treated with UV, red light and red light with UV on 5, 10, 15 and 20 d after harvesting. Data are means ± SE, n = 6 replicates. Different letters within each day indicate significant differences ($p \leq 0.05$).

Light source	NDVI	NAI	TSS (%)
Day 0			
Green tomato	0.66 ± 0.04	1.69 ± 0.25	4.35 ± 0.22
Day 5			
Control	0.66 ± 0.08^{bc}	1.63 ± 0.35^{a}	4.32 ± 0.10^{a}
UV	0.69 ± 0.01^{c}	1.71 ± 0.17^{a}	4.37 ± 0.22^{a}
Red light	0.52 ± 0.07^{a}	2.36 ± 0.54^{a}	4.90 ± 0.30^{b}
Red light + UV	0.61 ± 0.03^{b}	2.32 ± 1.21^{a}	4.80 ± 0.28^{b}
Day 10			
Control	0.67 ± 0.09^{b}	2.33 ± 0.15^{a}	4.61 ± 0.39^{a}
UV	0.68 ± 0.05^{b}	1.94 ± 0.40^{a}	4.76 ± 0.21^{ab}
Red light	0.00 ± 0.06^{a}	10.46 ± 0.67^{b}	4.95 ± 0.22^{ab}
Red light + UV	-0.03 ± 0.03^{a}	11.22 ± 0.89^{c}	5.09 ± 0.21^{b}
Day 15			
Control	-0.07 ± 0.01^{b}	12.06 ± 0.26^{b}	4.69 ± 0.22^{a}
UV	-0.07 ± 0.01^{b}	11.49 ± 0.23^{a}	4.78 ± 0.19^{ab}
Red light	-0.08 ± 0.01^{ab}	12.39 ± 0.44^{c}	5.08 ± 0.35^{b}
Red light + UV	-0.08 ± 0.01^{a}	12.42 ± 0.03^{c}	5.08 ± 0.18^{b}
Day 20			
Control	-0.06 ± 0.00^{c}	12.34 ± 0.04^{a}	4.41 ± 0.22^{a}
UV	-0.07 ± 0.01^{bc}	12.35 ± 0.06^{ab}	4.84 ± 0.63^{a}
Red light	-0.08 ± 0.01^{ab}	12.41 ± 0.05^{b}	4.72 ± 0.24^{a}
Red light + UV	-0.08 ± 0.01^{a}	12.42 ± 0.07^{b}	4.91 ± 0.39^{a}

3.2 Total soluble solids

At 5 d after harvesting, total soluble solids concentration (%) was significantly higher in tomato fruit exposed to red light and red light with UV as compared to control fruit and those treated with UV only. At 20 d after harvesting, there was no significant difference in TSS between the treatments (Table 1).

Table 2. Carotenoid, total flavonoid, total phenolic content and antioxidant activity of control tomato fruit, fruit treated with UV, red light and red light with UV on 5, 10, 15 and 20 d after harvesting. Data are means ± SE, n = 6 replicates. Different letters within each day indicate significant differences ($p \leq 0.05$).

Light source	Carotenoids concentration (mg k g^{-1})		Total flavonoid concentration (mg g^{-1})	Total phenolic concentration (mg g^{-1})	Antioxidant activities (mM g^{-1})	
	Lycopene	β-carotene			HAA	LAA
Day 0						
Green tomato	42.07 ± 5.12	44.16 ± 5.52	12.36 ± 0.87	5.31 ± 0.36	8.56 ± 0.29	9.41 ± 0.73
Day 5						
Control	44.22 ± 6.07^{a}	45.09 ± 8.05^{b}	11.16 ± 0.66^{bc}	3.51 ± 0.30^{a}	19.50 ± 0.66^{b}	9.37 ± 0.82^{a}
UV	54.61 ± 8.30^{a}	32.51 ± 5.44^{a}	6.24 ± 0.36^{a}	3.70 ± 0.22^{a}	20.69 ± 0.81^{c}	9.83 ± 0.82^{a}
Red light	40.92 ± 8.15^{b}	39.36 ± 5.34^{ab}	10.39 ± 0.93^{b}	5.05 ±0.56^{b}	17.13 ± 0.51^{a}	11.47 ± 0.82^{b}
Red light + UV	54.65 ± 8.21^{b}	67.19 ± 6.47^{c}	11.03 ± 1.51^{c}	5.49 ± 0.21^{c}	19.12 ± 0.93^{b}	12.44 ± 0.69^{c}
Day 10						
Control	131.92 ± 85.84^{a}	73.59 ± 5.39^{b}	12.04 ± 0.94^{b}	4.17 ± 0.24^{a}	21.39 ± 0.88^{b}	10.50 ± 0.24^{a}
UV	49.60 ± 9.62^{a}	38.64 ± 4.79^{a}	7.08 ± 0.63^{a}	4.15 ± 0.28^{a}	21.92 ± 0.75^{b}	10.42 ± 0.84^{a}
Red light	1049.08 ± 109.09^{b}	103.42 ± 7.37^{c}	13.38 ± 0.98^{c}	6.66 ± 0.26^{c}	19.81 ± 0.75^{a}	14.18 ± 0.81^{b}
Red light + UV	1280.18 ± 84.40^{c}	80.13 ± 7.40^{b}	12.05 ± 0.70^{b}	6.22 ± 0.26^{b}	22.10 ± 0.51^{b}	15.16 ± 0.52^{c}
Day 15						
Control	961.37 ± 159.00^{a}	69.11 ± 6.07^{b}	12.73 ± 0.47^{b}	4.92 ± 0.26^{b}	26.24 ± 0.76^{c}	12.97 ± 0.52^{a}
UV	855.14 ± 154.18^{a}	70.54 ± 8.92^{b}	8.11 ± 0.80^{a}	4.47 ± 0.37^{a}	23.49 ± 0.81^{b}	12.45 ± 0.36^{a}
Red light	1176.50 ± 156.44^{b}	51.20 ± 14.66^{a}	13.81 ± 0.58^{c}	7.08 ± 0.41^{c}	21.37 ± 0.72^{a}	16.91 ± 0.80^{b}
Red light + UV	1324.43 ± 97.58^{b}	45.29 ± 9.33^{a}	12.72 ± 0.97^{b}	7.28 ± 0.30^{c}	24.08 ± 0.88^{b}	16.29 ± 0.67^{b}
Day 20						
Control	871.16 ± 49.81^{a}	69.07 ± 3.94^{b}	14.88 ± 0.48^{b}	5.68 ± 0.53^{a}	22.82 ± 0.59^{a}	13.96 ± 0.95^{a}
UV	850.84 ± 31.36^{a}	52.47 ± 7.68^{a}	10.57 ± 0.62^{a}	5.72 ± 0.54^{a}	24.27 ± 0.79^{b}	13.22 ± 0.90^{a}
Red light	1507.17 ± 107.30^{c}	50.01 ± 11.04^{a}	15.10 ± 0.75^{b}	7.78 ± 0.19^{b}	27.82 ± 0.89^{c}	17.71 ± 0.84^{b}
Red light + UV	1413.91 ± 69.30^{b}	49.24 ± 9.40^{a}	15.18 ± 0.80^{b}	7.36 ± 0.27^{b}	28.83 ± 0.82^{d}	19.75 ± 0.90^{c}

3.3 Lycopene and ß-carotene

Lycopene concentration sharply increased between day 5 and 10 after harvesting in tomato fruit treated with red light and red light with UV, whereas control fruit and those radiated with UV light only did not show a significant increase in lycopene concentration until 15 d after harvest (Fig. 2A, Table 2). Concentration of β-carotene was highest 10 d post-harvest for control fruit, fruit treated with red light as well as a combination of red light and UV, with the red light radiation showing the highest β-carotene concentration among those three treatments (Fig. 2B, Table 2). After this peak,

β-carotene concentration sharply decreased in fruit treated with red light and red light with UV. Fruit treated with UV only had highest β-carotene concentrations 15 d after harvesting. After 20 d of harvesting, β-carotene concentration was highest for control fruit.

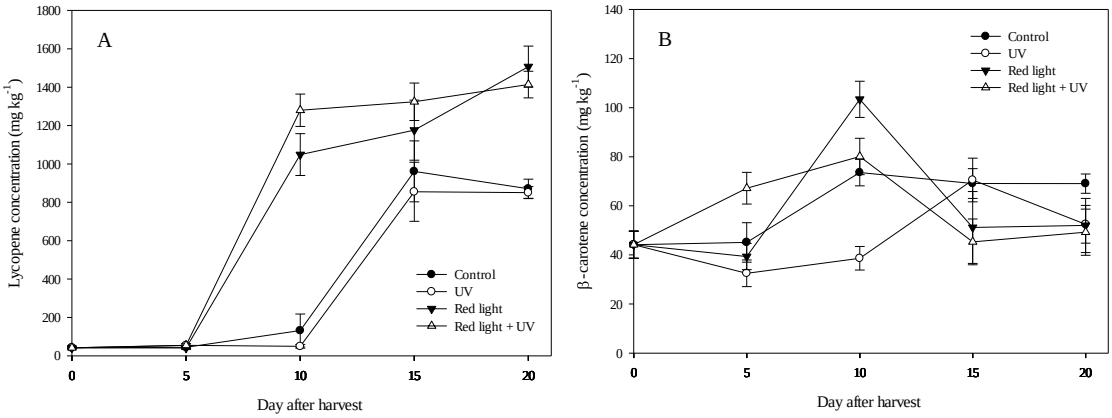

Figure 2. (A) Lycopene and (B) β-carotene concentration of control (closed circle) tomato fruit, fruit treated with UV (open circle), red light (closed triangle) and red light with UV (open triangle) on harvest day (0) and 5, 10, 15 and 20 d after harvesting. Means ± SE, n = 6.

3.4 Total flavonoids and phenolics

Fruit treated with UV light only showed a significant decrease in flavonoid concentration five days after harvesting and concentrations were lowest for all days until the end of the sampling period when compared to the other treatments (Fig. 3A, Table 2). Flavonoid concentration was highest for fruit radiated with red light 10 and 15 d after harvesting. There was no significant difference in flavonoid concentration between UV, red light and red light with UV exposure 20 d after harvesting. Control fruit and those treated with UV light only showed a significant decrease in total phenolic concentration at day 5 after harvesting and only slowly recovered to values obtained directly after harvesting until day 20 (Fig. 3B, Table 2). In contrast, total phenolic concentration increased sharply in fruit treated with red light 10 d after harvesting and peaked on day 20.

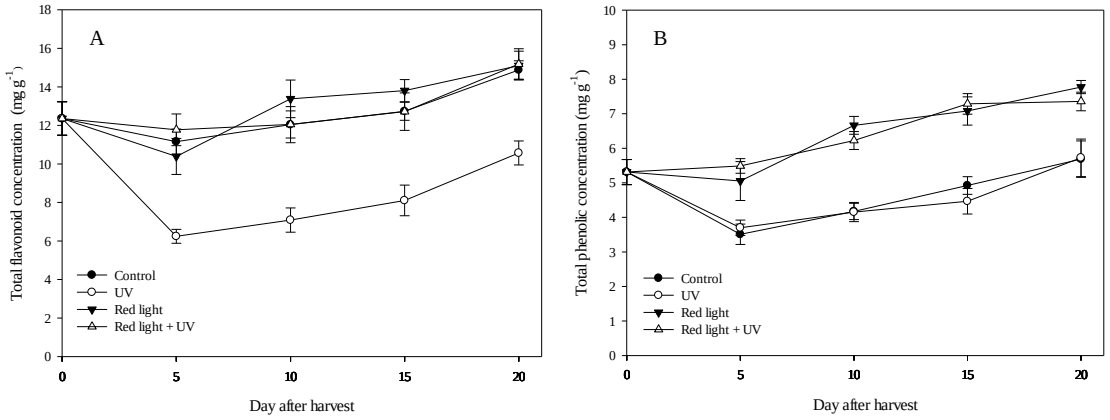

Figure 3. (A) Total flavonoid and (B) Total phenolic concentration of control (closed circle) tomato fruit, fruit treated with UV (open circle), red light (closed triangle) and red light with UV (open triangle) on harvest day (0) and 5, 10, 15 and 20 d after harvesting. Means ± SE, n = 6.

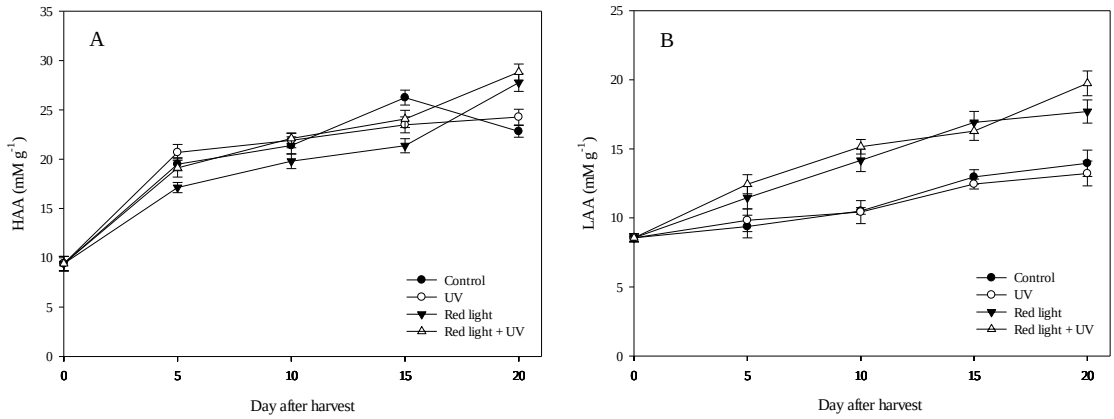

Figure 4. (A) Hydrophilic and (B) lipophilic antioxidant activity of control (closed circle) tomato fruit, fruit treated with UV (open circle), red light (closed triangle) and red light with UV (open triangle) on harvest day (0) and 5, 10, 15 and 20 d after harvesting. Means ± SE, n = 6.

3.5 HAA and LAA

All treatments increased HAA 5 d after harvesting (Fig. 4B, Table 2). However, fruit treated with red light and red light with UV showed highest LAA 20 d after harvesting compared to control fruit and those treated with UV light only. LAA was

highest for fruit treated with red light and red light with UV from day 5 after harvesting onwards (Fig. 4A, Table 2). All treatments increased HAA 5 d after harvesting (Fig. 4B, Table 2). However, fruit treated with red light with UV showed highest HAA and LAA 20 d after harvesting compared to control fruit and those treated with red light and UV light only.

4. Discussion

In our study, we aimed to shorten post-harvest ripening time and to increase levels of some health benefitting compounds in tomato fruit. Changes in both NDVI and NAI indicate that chlorophyll breakdown in the exocarp had ended earlier in fruit treated with red light (Fig. 1A, Table 1). In addition, Zude (2003) reports that red light had an effect on chlorophyll breakdown, and therefore led to a higher NAI in tomato fruit. Moreover, it proves that NAI represents a reasonable index for the non-destructive determination of lycopene concentrations. TSS includes the total sum of sugar acids (mainly citric and malic acid) and other components, such as phenols, amino acids, soluble pectin, Vitamin C and minerals (Balibrea et al., 2006; Kader, 2008). The TSS results presented here (Table 1) show that post-harvest treatments with UV light and red light with UV are a suitable option to increase TSS contents during 15 d of storage, possibly due to changes in the ratio of glucose/fructose and various organic acids (Balibrea et al., 2006; Kader, 2008).

Changes in both lycopene and ß-carotene contents during storage time (Fig. 2A, Table 2) are similar to those reported by Thiagu et al. (1993), who showed that ß-carotene increased up to the light-pink stage and declined afterwards during full and over-ripe stages of tomato ripening. Liu et al. (2009) report that lycopene concentration did not change significantly during the first 4 d of storage, independently of the treatment (untreated, red light, UV-C and sun light), but no further studies were carried out to examine the effects of a prolonged radiation. In addition, previous studies have shown that the total amount of carotenoids and lycopene significantly increases during fruit ripening (Ilahy et al., 2011). Furthermore, red light and its intensity had a positive effect on carotenoid synthesis in detached mature-green fruit (Thomas and Jen, 1975). Most likely, red light affects the biosynthesis of tomato carotenoids by enhancing the first step

of carotenogenesis by modulating the phytoene synthesis activity, which is an important control step of carotene biosynthesis (Schofield and Paliyath, 2005). However, to our knowledge, there are no reports about the effects of different light treatments on carotenoid concentrations during a prolonged post-harvest period. So far, previous studies have only examined the stimulating effect of UV on total phenolic compounds of fruit and vegetables. For example, irradiation with UV-B light increased the content of flavonols in the pericarp of apples (Bakhshi and Arakawa, 2006), most likely due to the DNA-damaging effects of UV light which occurs predominantly in the epidermal fruit tissues (Strack, 1997). This effect arises due to increased expression and activity of the enzyme phenylalanine ammonia lyase, a key enzyme in the production of phenylpropanoids, which leads to an increase of phenols, phytoalexins, and lignins (Ryalls et al., 1996). However, the results from the experiments presented here (Fig. 3A, Table 2) suggest that a combination of red light and UV is even more effective in increasing phenolic concentrations in tomato fruit after 10 and 15 days of storage. Some antioxidants are of hydrophilic nature (for example ascorbic acid and phenolics), whereas others, such as carotenoids are lipophilic (Zhou et al., 2012). Interestingly, hydrophilic antioxidant activity was between 25 and 50% higher than lipophilic antioxidant activity on day 20 after harvesting in our experiment (Fig. 4A and B, Table 2). Although Raffo et al. (2002) reported a similar trend for HAA during fruit maturation of the cherry tomato cv Naomi grown under greenhouse conditions. Also, different results were shown by Cano et al. (2003), who found that the HAA remained practically unchanged during ripening in the greenhouse-grown tomato. Furthermore, LAA was increased during tomato ripening, possibly due to changes in lycopene concentration (Cano et al., 2003).

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C Optimal red light irradiation time to increase health-promoting compounds in tomato fruit post-harvest[2]

1. Introduction

Recently, interest in nutritional value of food has rapidly increased due to associated positive health effects (Prasad and Chakravorty, 2015). Together with their derived products, tomatoes are one of the major food sources of carotenoids, providing roughly 80% of daily intake of lycopene, as well as folate, ascorbic acid, flavonoids, a-tocopherol and potassium in the Western diet (Willcox et al., 2003). These health promoting compounds of tomato fruit have mainly been attributed to the significant amount of natural antioxidants, especially lycopene (Ilić et al., 2012).

In the past decades, considerable work has been conducted to increase levels of carotenoids in tomatoes through breeding programs or ripening intervention technologies. In particular, during post-harvest storage irradiation with different light spectra has been tested (Alba et al., 2000; Liu et al., 2003; Rosati et al., 2000). Light is one of the most important environmental factors affecting the pigment metabolism of vegetables and fruit (Lado et al., 2015). As shown, light has a positive effect on nutritional quality of butterhead lettuces (*Lactuca sativa* L.) (Charles et al., 2018). Continuous light (around 35 μmol−2 s−1) can maintain the level of soluble sugars and ascorbic acid in post-harvest fresh-cut romaine lettuce (Zhan et al., 2013). In spinach leaves, the endogenous pool of some vitamins including ascorbic acid and folate is higher when leaves are stored under visible light than in the dark (Lester et al., 2010).

Therefore, the aim of this study was to investigate the effect of post-harvest red light radiation schemes (intermittent or continuous) on health promoting compounds in tomatoes. The guiding work hypothesis was that different red light irradiation time per day lead to a differentiated synthesis of heath promoting compounds in tomatoes. In order to reach the objectives, simple chlorophyll fluorescence ratio, lycopene, β-carotene, total

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phenolic concentration, total flavonoid concentration, as well as hydrophilic and lipophilic antioxidant activity were monitored during a storage period of 20 and 14 d in experiment 1 and 2, respectively.

2. Material and Methods

2.1. Tomato cultivation

Tomatoes (*Solanum lycopersicum* L.) of the cultivar Cappricia (RijkZwaan, De Lier, The Netherlands) were harvested from plants cultivated in a commercial-like greenhouse at the Campus Klein Altendorf research station (University of Bonn, Germany, 50°37′31.6″N 6°59′18.1″E, altitude 600 m). Green fruit classified according to the USDA maturity stage as Green Stage 1 (USDA, 2005) were harvested with calyx. In order to restrict potential influences of developmental and environmental factors, one healthy tomato fruit of standardized size was harvested from each truss. According to this scheme, the first fruit in each first truss from stub was picked. Afterwards, tomatoes were placed into plastic trays covered with aluminum foil (42 fruit per tray), ensuring that the fruit did not touch each other. The trays were stored in a custom-built climate chamber for 20 d in the first experiment (from now, Experiment 1) and 14 d in the second experiment (Experiment 2) under constant day/night temperatures (20 °C/19 °C) with variable day/night temperature-dependent relative humidity (RH, 75%/85%).

2.2 Light treatments

2.2.1 Experiment 1: Effects of duration of red light radiation on accumulation of health-promoting compounds

For this experiment, four different light treatments were used:

1) Darkness (control)
2) Continuous red light for 10 d, then fruit were kept in dark for 10 additional days
3) Continuous red light for 15 d, then fruit were kept in dark for 5 additional days
4) Continuous red light for 20 days

Fruit from treatments 2 and 3 were taken from the batch of treatment 4 (after having been irradiated for 10 and 15 d, respectively).

2.2.2 Experiment 2: Effects of intermittent or continuous red light on accumulation of health-promoting compounds

For this experiment, five different treatments were used:

1) Darkness (control)

2) Red light for 30 min per day

3) Red light for 6 h per day

4) Red light for 12 h per day

5) Continuous red light

In both experiments, tomatoes of the control treatment were placed in a box and kept in the dark (same temperature and RH conditions as in the climate chamber). For the red light treatments, tomatoes were irradiated with light emitting diode (LED) modules (Ushio Lighting Inc., Tokyo Japan) installed in the climate chamber. The LED settings (intensity and spectral composition) were controlled by the equipment specific-software. Red light was applied for the whole storage period (red light peak at 665 nm) which is equivalent to a photosynthetic available radiation (PAR) of 113 μmol m^{-2} s-1 (X1-2 SN4962 M RS232 optometer, Gigahertz-Optik GmbH, Germany). During the experimental time, tomatoes were carefully turned over every day (at 2 pm) to ensure light exposition of both fruit sides.

2.3 Fruit sampling and remittance determinations

Six tomatoes were sampled on the harvesting day (day 0) to investigate the fruit quality (stage) at the starting point of the experiment. The grading standards for fresh tomatoes set by the United States Department of Agriculture (USDA, 2005) were used in making a visual evaluation of the appearance and skin color of the fruit. For rating, the following scale was used:

1 = green, 100% green

2 = breaker, a noticeable break in color with less than 10% of color other than green

3 = turning, between 10 and 30% reddish color

4 = pink, between 30 and 60% reddish color

5 = light red, between 60 and 90% red

6 = red, more than 90% red

2.4 Firmness analysis

The hand-held penetrometer (HP Series Shore A, Bareiss, Oberdischingen, Germany) was used for firmness analysis. Three points on each fruit were evaluated in diameter around the equatorial zone of fruit per measurement day.

2.5 Simple chlorophyll fluorescence ratio (SFR)

The Multiplex® (FORCE-A, Orsay, France) is a hand-held, multiparametric fluorescence sensor that uses LED excitation light and filtered-photodiode detection, and was employed to estimate chlorophyll concentration, as described by Hoffman et al. (2015). For this purpose, the Simple Fluorescence Ratio with red excitation light was used. Fluorescence signals were detected in the red (680–690 nm) and far-red (720–755 nm) spectral regions with green excitation light. For fluorescence determinations, a hand-held multiparametric sensor (Multiplex®, Force A, Orsay, France) with a mask of 2 cm diameter and a constant distance of 0.10 m to the fruit surface was used. The sensor, its working principle, and the selected parameters are extensively described in the literature (Groher et al., 2018). Fluorescence signals were recorded through an aperture of 4 cm in diameter around the equatorial zone of fruit (three readings per sample and per measurement day). The selected parameters are calculated as follows:

SFR_R = FRF_R/RF_R

where FRF_R = Far-Red Fluorescence

RF_R=Red Fluorescence

2.6 Sample preparation for destructive analyses

As the experiment proceeded, predetermined intervals were specified at which six tomatoes would be selected at random from the main sample group. Tomatoes were cut into small pieces and kept at −25 °C prior to lyophilization (Gamma 1-16LSC, Christ, Osterode am Harz, Germany). These samples were then dried and ground into a powder prior to storage under conditions of darkness until further preparation, extraction procedures and lab analyses.

2.7 Extraction procedure

The aqueous 80% methanolic extraction (80% methanol [PubChem CID: 887] + 1.0% hydrochloric acid [PubChem CID: 313] [37%, Merck, Germany])) which followed the method employed by Ponmozhi et al. (2011) with minor alterations in order to determine for total phenolic content, total flavonoid content and hydrophilic antioxidant activity assay. Petroleum ether (60%) was used for lipophilic antioxidant activity, lycopene and β-carotene assay. 0.05 g of each lyophilized sample was placed in a 1.5 mL Eppendorf tube and then mixed with 1.0 mL of the respective solvent. The mixture was vortexed (Vortex ex-2 Gene, Scientific Industries, New York, USA) for 20 s followed by sonication for 5 min in an ultrasonic bath (Sonorex Super RK106, Bandelin electronic, Berlin, Germany). After that, the mixture was centrifuged at 15,682 g for 6 min to separate the supernatant (Eppendorf centrifuge 5415R, Hamburg, Germany). The experiment was performed in quadruplicate. Finally, the supernatant volume was adjusted to 5 mL with the solvent and filtrated through a 0.20 μm membrane (regenerated cellulose, Phenomenex, Torrance, California, USA).

2.8 Lycopene and β-carotene

Lycopene and β-carotene concentrations were investigated by using high performance liquid chromatography (HPLC, Agilent 1260-series, Waldbronn, Germany) according to the method described by Olives et al. (2006). For quantification, the HPLC was equipped with a reverse phase system with an autosampler and a Eurospher100-C18

column (250 x 4 mm, 5 μm packing material, KNAUER Wissenschaftliche Geräte GmbH, Germany). The chosen setting for the detector was 475 nm, and the column (temperature controlled at 30 °C) was eluted with a mobile phase containing methanol/ACN (PubChem CID: 6342) (90/10 v/v) with triethylamine (TEA) (PubChem CID: 8471) 9 μM. The results were analysed by using Chemstation software. Lycopene (PubChem CID: 446,925) and β-carotene (PubChem CID: 5,280,489) were used as the standards for a linear calibration curve between 0.02 and 20 μg mL^{-1}. Lycopene and β-carotene were expressed as milligram per kilogram dry mass.

2.9 Total phenolic concentration

Total phenolic content was determined by using the Folin-Ciocalteu method as described by Chen et al. (2015), with slight modifications. Briefly, a volume of 0.2 mL of the methanolic extract was adjusted to 0.5 mL with water and then mixed with 0.5 mL of 1:10 diluted Folin-Ciocalteu reagent. The mixture was allowed to incubate at room temperature for 4 min prior to the addition of 1.5 mL of saturated sodium carbonate (PubChem CID: 516,892) solution (75 g L^{-1}) and was further incubated for 120 min. The absorbance of the mixture was measured at 760 nm by using a spectrophotometer (Lambda 35 UV/Vis spectrophotometer, PerkinElmer, Boston, Massachusetts, USA) and the respective solvent was used as blank. Gallic acid (PubChem CID: 370) was used as the standard for a linear calibration curve between 50 and 500 mg L^{-1}. The total phenolics content was expressed as milligram of gallic acid equivalents (mg GAE g^{-1}) dry mass.

2.10 Total flavonoid concentration

Total flavonoid content was determined by using the aluminum chloride colorimetric method described previously (Chen et al., 2015) with slight modifications. Briefly, a volume of 1 mL of the methanolic extract was added to 0.1 mL of 5% sodium nitrite (PubChem CID: 23,668,193) solution. The mixture was allowed to incubate for 6 min, then 0.1 mL of 10% aluminum chloride (PubChem CID: 24,012) solution was added and the mixture was further incubated for 5 min, prior to adding 0.1 mL of 1 M sodium hydroxide (PubChem CID: 14,798) and was vortexed for 10 s. After adding 1.7 mL of

water and 30 min of incubation, the absorbance was measured at 510 nm using a spectrophotometer (Lambda 35 UV/Vis spectrophotometer, PerkinElmer, Boston, Massachusetts, USA). The respective solvent was used as blank. Quercetin (PubChem CID: 5,280,804) was used as the standard for a linear standard curve between 100 and 1000 mg L^{-1}. The total flavonoids content was expressed as milligram of quercetin equivalents (mg QAE g^{-1}) dry mass.

2.11 Hydrophilic and lipophilic antioxidant activity

The hydrophilic and lipophilic antioxidant activities were investigated by using the 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS+, PubChem CID: 5,360,881) decolorization method as described by Thaipong et al. (2006) with slight modifications. The stock solutions included 8 ml of 7 mM ABTS solution (0.0384 g of ABTS+ adjusted to 10 mL of water) and 12 ml of 2.45 mM potassium persulfate (PubChem CID: 24,412) solution (0.0166 g of K2S2O8 adjusted to 25 mL of water), which were allowed to react for 12 h at room temperature in the dark. Before use, the solution was then diluted with methanol to obtain an absorbance between 0.8 and1.0 units at 734 nm by using the spectrophotometer (Lambda 35 UV/Vis spectrometer, PerkinElmer, Boston, Massachusetts, USA). A volume of 80 µL of extract was added to 1960 µL of the ABTS solution. The absorbance was read after incubation for 2 h in dark conditions. The percentage of inhibition of free radical ABTS was generated from the following equation. % of inhibition = ((Abs control-Abs sample) /Abs control) X 100 Trolox (PubChem CID: 6,541,354) was used as the standard and the results were expressed in mMTrolox equivalents (TE g^{-1}) dry mass.

2.12 Statistical analysis

Statistical analyses were performed by using SPSS 22.0. Analysed data is expressed as means ± SE. Significant differences between treatments for all parameters were analysed by using a one-way ANOVA. A Tukey HSD test was conducted to establish the differences among mean values. The threshold p-value chosen for statistical significance was $p \leq 0.05$. Graphs were generated by using SigmaPlot12.

3. Results

3.1 Experiment 1: Effect of duration of continuous red light radiation time on health-promoting compounds

3.1.1 Firmness

Fruit treated with red light for 20 d showed a very rapid loss of firmness between day 5 and 12 after harvesting, while firmness loss of control fruit was slower (Fig. 1). There was no significant difference in firmness between fruit treated with red light for 20 d and red light for 10 d between day 12 and 14 after harvesting. Lowest firmness was recorded from day 16–20 after harvesting in fruit treated with red light for 15 d and red light for 20 d.

3.1.2 Chlorophyll content indicator, SFR_R

SFR_R of fruit treated with red light for 20 d and control fruit sharply decreased from day 5–12 after harvesting (Fig. 2). Furthermore, there was a gradual decrease in SFR_R in tomatoes treated with red light for 20 d and red light for 15 d. The significantly lowest SFR_R value was recorded for fruit treated with red light for 20 d from day 12 onwards.

3.1.3 Lycopene and ß-carotene

Lycopene and β-carotene concentration in fruit treated with red light for 20 d drastically increased from day 5–10 after harvesting (Fig. 3A and B). Lycopene concentration was significantly affected when tomatoes were treated with red light for 20 d from day18 after harvesting (Fig. 3A). At the end of the storage period, fruit treated with red light for 20 d showed significantly highest lycopene concentration compared to all other treatments. β-carotene concentration of fruit treated with red light for 20 d and red light for 10 d was significantly different on 12 and 14 d after harvesting compared to the other treatments (Fig. 3B). On 16 and 18 d after harvesting, β-carotene of fruit treated with red

light for 20 d and red light for 15 d showed significantly higher concentrations compared to red light for 10 d and the control fruit. Twenty d after harvesting, β-carotene concentration was significantly higher in fruit treated with red light as compared to all other treatments.

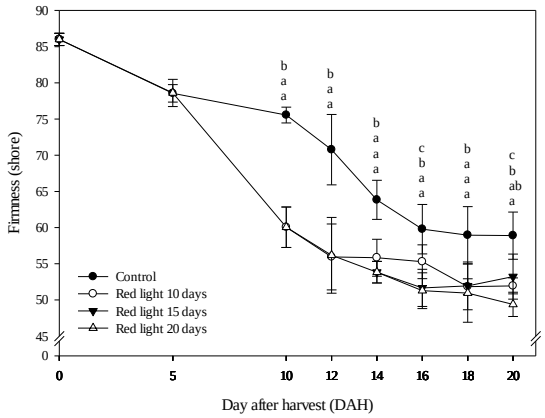

Figure 1. Fruit firmness of control (closed circle) tomatoes, tomatoes treated with red light for 10 d (open circle), red light for 15 d (closed triangle) and red light for 20 d (open triangle), on harvest day (0) and 5, 10, 12, 14, 16, 18 and 20 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

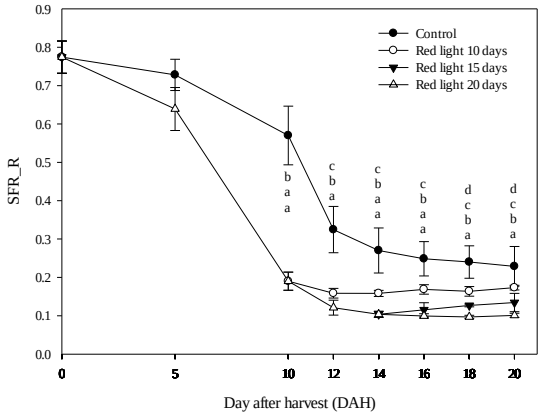

Figure 2. SFR_R of control (closed circle) tomatoes, tomatoes treated with red light for 10 d (open circle), red light for 15 d (closed triangle) and red light for 20 d (open triangle) on harvest day (0) and 5, 10, 12, 14, 16, 18 and 20 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

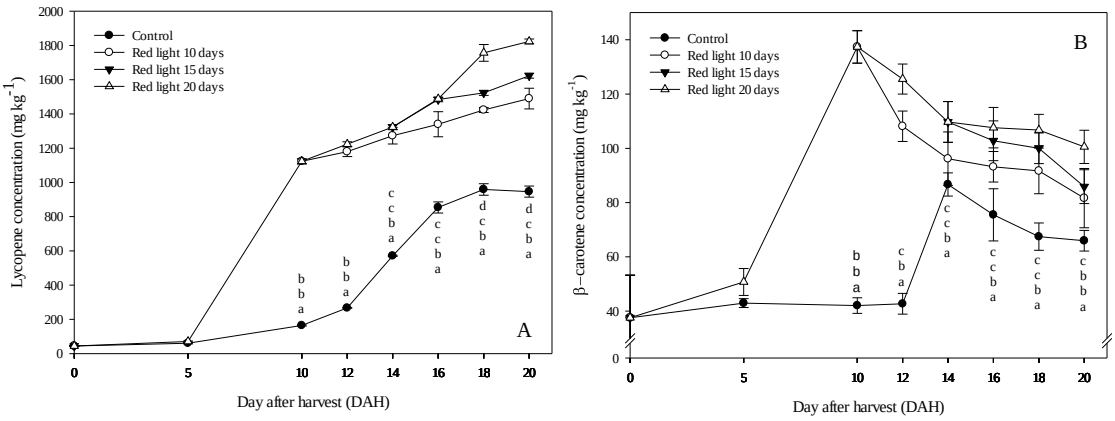

Figure 3. (A) Lycopene and (B) β-carotene concentration of control (closed circle) tomatoes, tomatoes treated with red light for 10 d (open circle), red light for 15 d (closed triangle) and red light for 20 d (open triangle) on harvest day (0) and 5, 10, 12, 14, 16, 18 and 20 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.1.4 Total flavonoid and phenolic concentration

Total flavonoid and phenolic concentration of tomatoes exposed to red light for 20 d sharply increased from day 5 and 10 after harvesting, respectively (Fig. 4A and B). Red light for 20 d stimulated total flavonoid concentration as those fruit showed significantly highest contentrations during storage time compared to the other treatments. Total phenolic concentration was significantly higher in fruit treated with red light for 20 d from day 5 after harvesting onwards, although there was no significant difference in total phenolic concentration between tomatoes treated with red light for 20 d and red light for 15 d.

3.1.5 Hydrophilic and lipophilic antioxidant activity

Tomatoes exposed to red light for 10, 15 and 20 d had a rapid increase in HAA from day 0–5 after harvesting, and in LAA from day 5–14 after harvesting (Fig. 5A). HAA was significantly higher in fruit treated with red light for 20 d on day 12, 14 and 20 after harvesting. Furthermore, highest values of LAA were recorded for tomatoes treated with red light for 20 d on day 12, 14, 16 and 20 after harvesting (Fig. 5B).

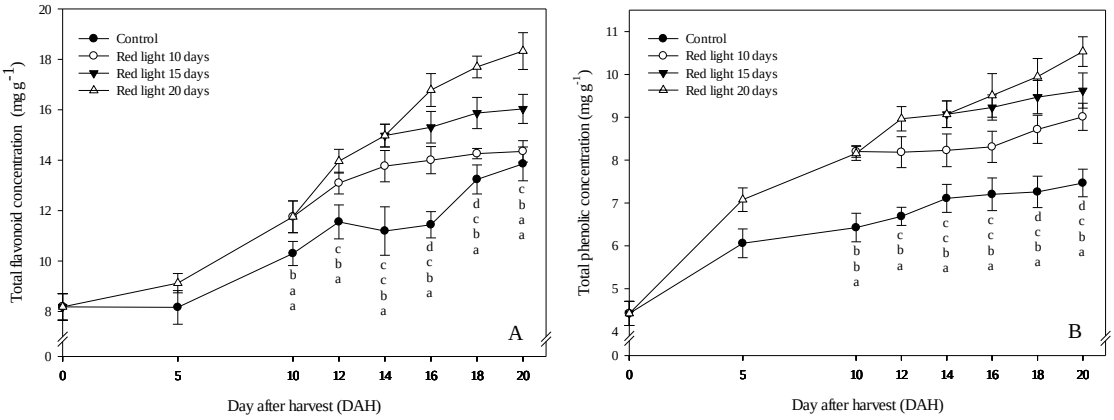

Figure 4. (A) Total flavonoid and (B) total phenolic concentration of control (closed circle) tomatoes, tomatoes treated with red light for 10 d (open circle), red light for 15 d (closed triangle) and red light for 20 d (open triangle) on harvest day (0) and 5, 10, 12, 14, 16, 18 and 20 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

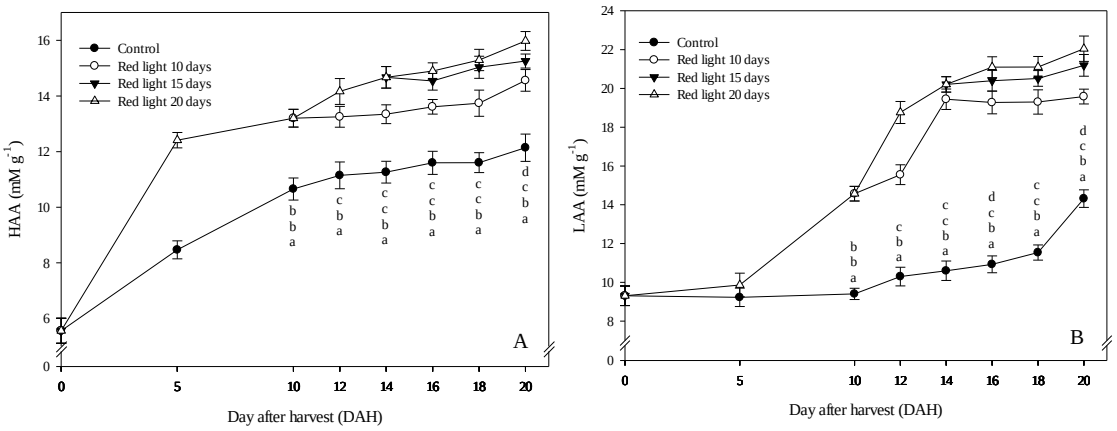

Figure 5. (A) Hydrophilic and (B) lipophilic antioxidant activity of control (closed circle) tomatoes, tomatoes treated with red light for 10 d (open circle), red light for 15 d (closed triangle) and red light for 20 d (open triangle) on harvest day (0) and 5, 10, 12, 14, 16, 18 and 20 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.2 Experiment 2: Effect of intermittent or continuous red light on health promoting compounds

3.2.1 Fruit firmness

Fruit firmness in all experimental treatments slowly decreased until day 5 after harvesting during storage time (Fig. 6). Thereafter, continuous red light led to a fast decline in firmness, and values were lowest for this treatment until the end of the storage period. Highest pulp firmness throughout the treatment period was recorded for fruit radiated with red light for 30 min.

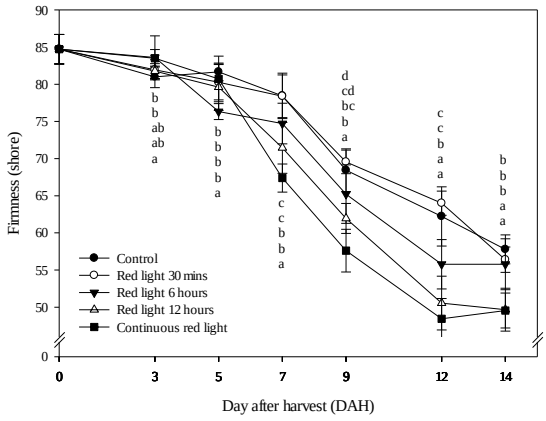

Figure 6. Fruit firmness of control (closed circle) tomatoes, tomatoes treated with red light for 30 minutes (open circle), red light for 6 hours (closed triangle), red light for 12 hours (open triangle) and continuous red light (open square) on harvest day (0) and 3, 5, 7, 9, 12 and14 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.2.2 Chlorophyll content indicator, SFR_R

SRF_R values decreased from day 3 after harvesting onwards for all treatments, apart from untreated fruit, which showed a small increase in the SFR_R index from day 9–12 after harvesting and decreased again on day 14 after harvesting (Fig. 7). Five d after harvesting, SFR_R was significantly highest in tomatoes continuously exposed to red light. There was a rapid decrease in SFR_R between day 5 and 9 after harvesting for all

treatments. In addition, SRF_R was significantly higher in tomatoes treated with continuous red light and red light for 12 h from day 9 after harvesting onwards as compared to all other treatments.

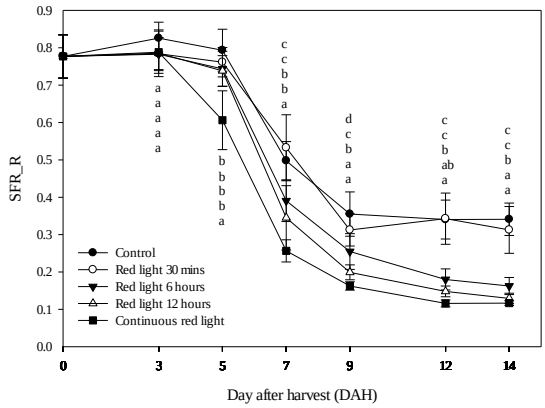

Figure 7. SFR_R of control (closed circle) tomatoes, tomatoes treated with red light for 30 minutes (open circle), red light for 6 hours (closed triangle), red light for 12 hours (open triangle) and continuous red light (open square) on harvest day (0) and 3, 5, 7, 9, 12 and14 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.2.3 Lycopene and ß-carotene

Lycopene concentration sharply increased between day 5 and 12 after harvesting in tomatoes treated with red light continuously and for 12 h, while fruit treated with red light for 6 h, 30 min and control only showed slightly increased lycopene concentration (Fig. 8A). Lycopene concentration was highest in fruit treated with continuous red light from day 7 after harvesting onwards. There was no significant difference in lycopene concentration between fruit treated with red light for 6 h, 30 min and control. Three days after harvesting, β-carotene concentration was significantly higher in tomatoes exposed to continuous red light and continued to drastically increase until 12 d after harvesting (Fig. 8B). In the other treatments, tomatoes also showed an increase in ß-carotene concentration until day 12 after harvesting; however, it was not nearly as pronounced as in fruit radiated continuously. After this peak, β-carotene decreased in all treatments.

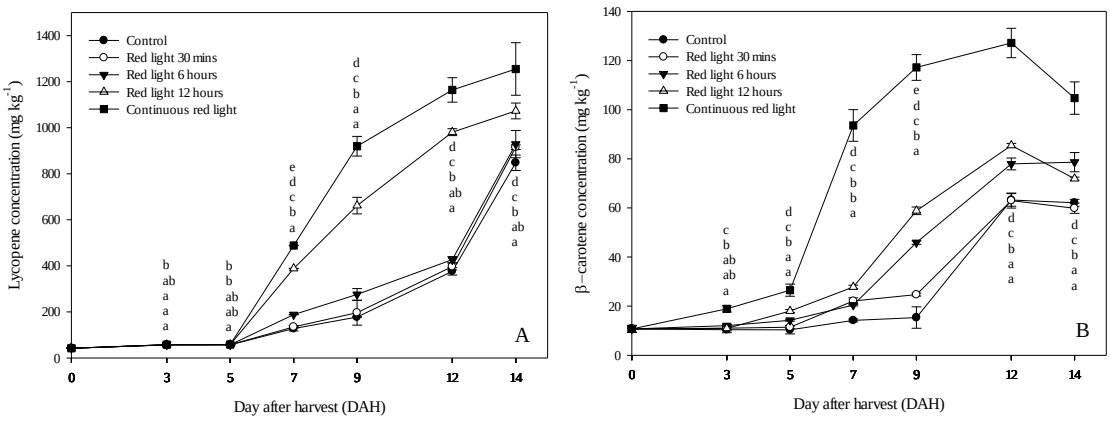

Figure 8. (A) Lycopene and (B) β-carotene concentration of control (closed circle) tomatoes, tomatoes treated with red light for 30 minutes (open circle), red light for 6 hours (closed triangle), red light for 12 hours (open triangle) and continuous red light (open square) on harvest day (0) and 3, 5, 7, 9, 12 and 14 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.2.4 Total flavonoid and phenolic concentration

Fruit treated with red light for 6 h or 12 h and continuous red light showed a significant increase in flavonoid concentration from day 5 after harvesting onwards compared to the control treatment (Fig. 9A). This gap was most pronounced 9 d after the treatment had started. Flavonoid concentration was highest for fruit exposed to continuous red light on day 9, 12 and 14 after harvesting. Even though total phenolic concentration increased in all treatments throughout the experimental period, it was highest in fruit treated with continuous red light from day 3 after harvesting onwards (Fig. 9B).

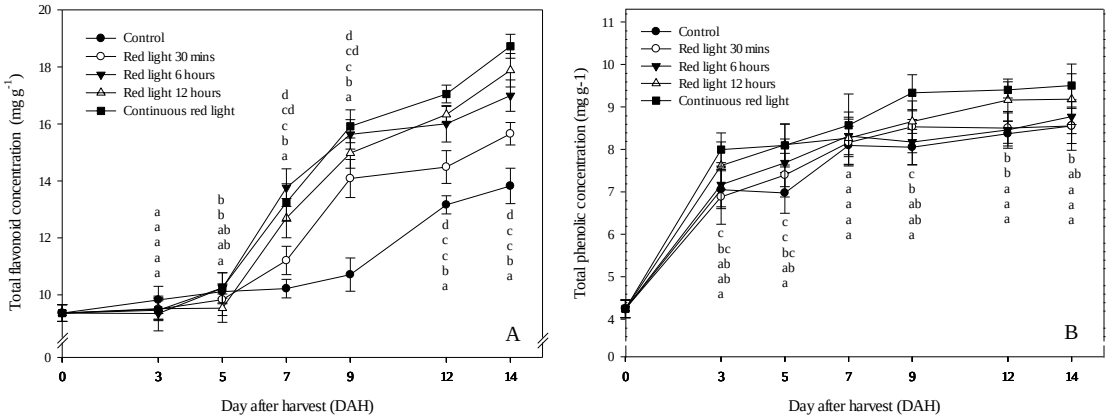

Figure 9. (A) Total flavonoid and (B) total phenolic concentration of control (closed circle) tomatoes, tomatoes treated with red light for 30 minutes (open circle), red light for 6 hours (closed triangle), red light for 12 hours (open triangle) and continuous red light (open square) on harvest day (0) and 3, 5, 7, 9, 12 and 14 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

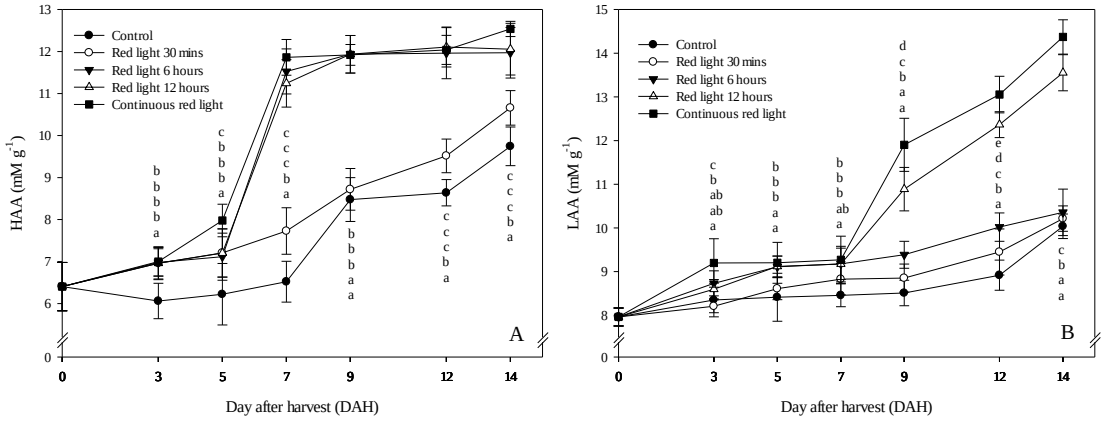

Figure 10. (A) Hydrophilic and (B) lipophilic antioxidant activity of control (closed circle) tomatoes, tomatoes treated with red light for 30 minutes (open circle), red light for 6 hours (closed triangle), red light for 12 hours (open triangle) and continuous red light (open square) on harvest day (0) and 3, 5, 7, 9, 12 and 14 d after harvesting. Means ± SE, n = 6, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.2.5 Hydrophilic and lipophilic antioxidant activity

All treatments increased HAA from day 3 after harvesting onwards (Fig. 10A). However, fruit stored under red light for 12 h and 6 h and continuous exposure had significantly higher HAA from day 7–14 after harvesting as compared to control fruit and those treated with red light for 30 min. LAA concentration was significantly higher on day 9 after harvesting for continuous red light irradiation and sharply increased from day 7 to 14 after harvesting compared to the other treatments (Fig. 10B). Tomatoes treated with red light for 30 min, 6 h and control only showed slight increases in LAA concentration throughout the treatment period.

4. Discussion

This study examined the impact of red light irradiation on the post-harvest ripening process and quality of tomatoes. In a first approach, red light was applied for different storage periods (10 d, 15 d and 20 d red light; Experiment 1). In a next approach, the effect of varying red light periods during the day was studied (30 min, 6 h, 12 h and continuous red light; Experiment 2).

The simple chlorophyll fluorescence ratio (SFR_R) was employed to estimate chlorophyll concentration in tomato fruit in these experiments. Experiment 1 proved that significantly lowest SFR_R values were seen in fruit treated with red light for 20 d (until the end of the storage period, 20 d after harvesting, Fig. 1). Results of experiment 2 again suggest that continuous red light was most effective, as it led to a sharp decrease in SFR_R values already from day 5–7 after harvest (Fig. 6). Light quality is one of the most important environmental factors affecting the pigment metabolism of vegetables and fruit (Yuan et al., 2017), and the typical color changes during tomato ripening from green to red are associated with chlorophyll breakdown and the synthesis of carotenoid pigments due to the transformation of chloroplasts to chromoplasts (Toivonen and Brummell, 2008). In addition, different wavelengths have been reported to affect fruit color and maturation in different plants (Lopez et al., 2007). Tao et al. (2003) investigated the influence of sun light on chlorophyll levels in citrus fruit, where the chlorophyll content decreased quickly, which resulted in an earlier color development. Results presented here

suggest a similar effect of continuous red light on chlorophyll breakdown and pigment synthesis in tomato fruit.

Tomatoes exposed to continuous red light accumulated significantly more lycopene and β-carotene during storage time in comparison with the control tomatoes and tomatoes treated with red light for a lesser amount of time (Fig. 3A and B, 8 A and B). These fruit also developed a more intensive color which led to an improved visual quality (data not shown). Changes in both lycopene and β-carotene are similar to those reported by Panjai et al. (2017), who showed that lycopene and β-carotene increased significantly during the first 5 d of storage of continuous red light treatment in tomato fruit. Lycopene is considered the predominant carotenoid in tomatoes (80–90%), followed by β-carotene (5–10%) (Lenucci et al., 2006). Lycopene synthesis may increase by changing the lighting conditions of plants during fruit ripening (Pék and Helyes, 2010; Pék et al., 2011). The amount of light received by tomatoes after the onset of ripening appears to be a very important factor to increase lycopene biosynthesis (Jarquin-Enriquez et al., 2013; Gao et al., 2011). In addition, changes in photoperiod or day length increased lycopene content in tomato fruit produced in greenhouses during three different sampling periods (Jarquín-Enríquez et al., 2013). Moreover, fruit that received a greater amount of light accumulated more lycopene, developed a better color and had a better visual quality (Jarquín-Enríquez et al., 2013). Furthermore, is has been shown that accumulation of phytonutrients (including lycopene) is strongly affected by the intensity, duration and quality of light after harvest (Dorais, 2007; Dorais et al., 2008). Therefore, it seems likely that the longer the radiation lasts, the more lycopene is accumulated, and radiation for 30 min per day is not enough to stimulate increased lycopene synthesis.

Total flavonoid and phenolic concentrations in tomatoes exposed to red light for 20 d increased more quickly than in other treatment groups (Fig. 4A and B). In addition, total flavonoid concentration increased sharply in all red light treatments but only fruit which were treated with continuous red light showed highest flavonoid concentration from day 9 after harvesting onwards (Fig. 8A). Moreover, fruit treated with continuous red light showed the highest concentration of total phenolics during storage time (Fig. 8B). In general, light intensity and quantity favor the production of flavonoids (quercetin) during storage (Agati et al., 2013). Moreover, red wavelength (625–700 nm) supplements might lead to increased phenolic compounds in green vegetables (Olle and

Virsile, 2013). These studies are in accordance with the results presented here, suggesting that continuous red light is most effective in increasing total flavonoid and phenolic concentrations in tomato fruit.

Hydrophilic antioxidant activity (HAA) concentration was highest in fruit treated with red light radiation for 20 d and continuously compared to the other treatments (Fig. 5A and 10 A). Changes in antioxidant activity increased during ripening time by red light treatment. LAA concentration followed the same pattern as lycopene, where fruit treated with continuous red light accumulated more LAA during storage time (Fig. 5B and 10B). This result was expected as lycopene is a lipophilic antioxidant compound. In addition, Darwish et al. (2015a) have demonstrated that a short duration cycle of light/darkness applied for a few d increased antioxidant activities in tobacco leaves, and this effect was maintained several weeks after returning to normal light conditions. In addition, Charles et al. (2018) proposed a residual effect of light since the positive outcome can be maintained even when products are stored back in darkness. These studies are similar with the results presented here, confirming that red light still has an effect on antioxidative compounds even when tomatoes are stored back in darkness after red light irradiation.

Changes in firmness throughout the post-harvest period were significantly affected by red light radiation (Fig. 1 and Fig. 6), and fruit treated with red light irradiation for 24 h showed the fastest softening during the storage period, while changes in other treatments were less pronounced. Weight loss and firmness are physical parameters commonly monitored during storage due to their significant impact on tomato fruit appearance, which is one of the most important factors for consumer satisfaction (Cozmuta et al., 2016). It has been observed that firmness is greatly affected by internal changes (turgor pressure, dissolved free sugars level), but can also change through environmental conditions, such as temperature, humidity and light (Cozmuta et al., 2016). During tomato ripening, a softening process occurs on account of the activity of several enzymes that alter the structural components of the cell wall and diminish cell adhesion (Toivonen and Brummell, 2008). Cano et al. (2003) suggested that the ripening of tomatoes is characterized by softening of the fruit, degradation of chlorophyll, and increases in respiration rate, ethylene production, and the synthesis of acids, sugars and lycopene. In addition, previous studies have shown that radiation with red light was

sufficient to trigger full tomato ripening at 10 d after harvesting (Panjai et al., 2017). Hence, changes in firmness in green stage 1 tomato fruit was greatly affected by continuous red light radiation.

5. References

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D Effect of post-harvest irradiation with red light on epidermal color and carotenoid concentration in different parts of tomatoes[3]

1. Introduction

As climacteric fruit**,** tomatoes continue to ripen even after harvest. During ripening, the green pigment chlorophyll breaks down and carotenoids are synthesized (Liu et al., 2009). Therefore, considerable work has been conducted to accelerate or even increase levels of these compounds in tomatoes through ripening intervention technologies during post-harvest storage (Rosati et al., 2000).

Several studies have shown that the concentration of lycopene and β-carotene in fresh tomatoes depend on diverse factors such as cultivars (Valverde et al., 2013), soil and climate conditions (Kapoulas et al., 2011; Vinha et al., 2012) as well as degree of ripening and post-harvest storage conditions (Minoggio et al., 2003). Modification of light intensity and/or quality is particularly promising because of the pivotal role of light influencing main metabolic processes in the biosynthesis of phytochemical compounds (Castagna et al., 2013). Early studies have indicated that carotenoid biosynthesis in tomato fruit is induced by red light radiation applied post-harvest (Panjai et al., 2017). Alba et al. (2000) reported that red light treatments (six 40W Gro-lux lamps) increased lycopene accumulation 2.3-fold in tomatoes. Other studies have shown that red light treatment increases the carotenoid content and red color of tomatoes (Liu et al., 2009). Carotenoids, particularly lycopene and β-carotene, represent the primary components of ripe fruit pigmentation (deep red and orange color) in the tomato pericarp. These carotenoids largely influence the quality perception of fresh tomatoes (Liu et al., 2009).

The majority of the flavonols in tomatoes are located in the epidermis (Stewart et al., 2000). It has also been shown that the skin contains significantly higher levels of phenolics, flavonoids, lycopene, ascorbic acid and antioxidant activity than pulp and seed

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fractions (Shi and Le, 2000; Toor and Savage, 2005). This antioxidative capacity is given by constituents such as lycopene, phenolics, flavonoids, ascorbic acid, and vitamin E (Leonardi et al., 2000). Furthermore, due to its strong color and non-toxicity, lycopene extract from tomato peel is intended for use as a food colorant. It provides different color shades, ranging from yellow to red, based on reflectance of the natural synthetic lycopene structure. To increase the nutritional value of tomato pastes in order to enhance carotenoids intake, studies have targeted the peel enrichment of tomatoes with valuable metabolites (Reboul et al., 2005). Hence, in the fruit, the accumulation of the pigments in the epidermis prevents direct incidence of harmful light on the pulp, another factor that has been linked to the deterioration of bioactive compounds (Lee and Chen, 2002; Peng et al., 2008).

So far, most studies have focused either on the effect of pre-harvest or of post-harvest storage conditions on bioactive compounds in tomatoes. However, information about how concentrations might be influenced in the different fruit fractions, especially under red light radiation, has still not yet been clarified. Therefore, the objective of this study was to investigate the onset of first spectral alterations on skin reflectance and external fruit color, altered contents of beneficial compounds, such as lycopene, β-carotene, total phenolic and total flavonoid concentrations of the outer and inner parts, and the chlorophyll fluorescence ratio, of green stage-1 tomatoes during 21 days of post-harvest storage.

2. Materials and methods

2.1 Tomato cultivation

Tomato plants (*Solanum lycopersicum*) F1 hybrid ‘Lyterno’ (Rijk Zwaan Distribution B.V., The Netherlands) were grown in a commercial-like greenhouse at the Campus Klein-Altendorf research station (University of Bonn, Germany, 50°37'31.6"N 6°59'18.1"E, altitude 600 m). One healthy green tomato fruit of standardized size was harvested with sepal and pedicel from each truss, then classified according to the USDA maturity stage as Green Stage 1 (USDA, 2005). In order to restrict potential influences of developmental and environmental factors, one healthy tomato fruit of pre-defined size

was harvested from each truss (always from the same position). Afterwards, tomatoes were placed into plastic trays covered with aluminum foil (42 fruit per tray), ensuring that the fruit did not touch each other. The trays were stored in a custom-built climate chamber for 21d under constant day and night temperatures (20.6 ± 1.7 and 19.2 ± 0.8 ^{o}C) with variable day and night temperature-dependent relative humidity (75.5 ± 1.2 and 85 ± 1.8 %).

2.2 Light treatments

For this experiment, four different light treatments were conducted: 1) Darkness; 2) Red light for 12 hours per day; 3) Continuous red light for 15 d, then fruit were kept in dark for 6 additional days; 4) Continuous red light for 21 days. Fruit from treatment 3 were taken from the batch of treatment 4 (after having been irradiated for 15 d).

Tomatoes of the control treatment group were placed in a box and kept in the dark (same temperature and RH conditions as in the climate chamber). For the red light treatments, tomatoes were irradiated with light emitting diode (LED) modules (Ushio Lighting Inc., Tokyo Japan) installed in the climate chamber. The LED settings (intensity and spectral composition) were controlled by the equipment-specific software. Red light was applied for the whole storage period (red light peak at 665 nm) which is equivalent to a photosynthetic active radiation (PAR) of 113 µmol m^{-2} s^{-1} (X1-2 SN4962M RS232 optometer, Gigahertz-Optik GmbH, Germany). During the experimental time, tomatoes were carefully turned over every day (at 2 pm) to ensure light exposition of both fruit sides.

2.3 Fruit sampling and emittance determinations

Five tomatoes of the green stage were sampled on the harvesting day (day 0) to investigate the fruit quality at the starting point of the experiment. The grading standards for fresh tomatoes set by the United States Department of Agriculture (USDA, 2005) which is set to six maturity stages (green, breaker, turning, pink, light red, and red) were used in making a visual evaluation of the appearance and epidermal color of the fruit.

2.4 Simple Chlorophyll Fluorescence Ratio (SFR)

The Multiplex® (FORCE-A, Orsay, France) is a hand-held, multi-parametric fluorescence sensor that uses LED excitation light and filtered-photodiode detection, and was employed to estimate chlorophyll concentration as described by Hoffman et al. (2015). For this purpose, the Simple Fluorescence Ratio with red excitation light was used. Fluorescence signals were detected in the red (680–690 nm) and far-red (720–755 nm) spectral regions with red excitation light. For fluorescence determinations, a hand-held multiparametric sensor (Multiplex®, Force A, Orsay, France) with a mask of 4 cm diameter and a constant distance of 0.10 m to the fruit surface was used. The sensor, its working principle and the selected parameters are extensively described in the literature (Groher et al., 2018). Fluorescence signals were recorded through an aperture of 4 cm in diameter around the equatorial zone of the fruit (three readings per sample and per measurement day). The selected parameter (index) is calculated as followed:

SFR_R = FRF_R/RF_R,

where FRF_R = Far-Red Fluorescence

RF_R = Red Fluorescence

2.5 External fruit color

Tomato surface color values were measured using a portable spectrophotometer (CM-700d, Konica Minolta Inc., Tokyo, Japan) and readings at 3 points on the equator of each fruit were recorded. A sensing area of 7 mm^2 was used to read the L*, a*, and b* values of the CIELAB model. Hunter a*, b* and L* values were obtained, and color was expressed as the a*/b* ratio. The a*/b* ratio is the ratio of yellow–red to blue–green components of color and represents the color index related to color variation during tomato ripening (Liu et al., 2009).

2.6 Sample preparation for destructive analyses

Sampling was performed on initial harvest every day, then every second day after 5 days of harvest (day 0, 5, 7, 9, 11, 13, 15, 17, 19 and 21). For this purpose, five fruits were randomly chosen from each treatment. After analysis of above-mentioned optical and sensor-based properties, the outer part (epicarp and mesocarp) and inner part (endocarp and seed) of the fruit were carefully separated using a sharp knife. The weights of the whole tomatoes and their parts were recorded (*data not shown*). The tomato parts were cut into small pieces and kept at -25 °C for lyophilization (Gamma 1-16LSC, Christ, Osterode am Harz, Germany). These samples were then dried and ground into a powder prior to storage under conditions of darkness until further preparation, extraction procedures and lab analyses.

2.7 Extraction procedure

The methanolic extraction procedure was carried out according to Ponmozhi et al. (2011) which was modified by Panjai et al. (2017). The aqueous 80% methanolic extraction (80 % methanol [PubChem CID: 887] + 1.0 % hydrochloric acid [PubChem CID: 313] [37 %, Merck, Germany])) was done in order to determine total phenolic content, total flavonoid content and hydrophilic antioxidant activity assay. Petroleum ether (60 %) was used for lipophilic extraction (Panjai et al., 2017) in order to determine lipophilic antioxidant activity lycopene and β-carotene assay. About 0.05 g and 0.10 g of each lyophilized sample were placed in a 1.5 mL Eppendorf tube and then mixed with 1.0 mL of the respective solvent for lipophilic and methanolic extraction respectively. The mixture was vortexed (Vortex ex-2 Gene, Scientific Industries, New York, USA) for 20 sec followed by sonication for 5 min in an ultrasonic bath (Sonorex Super RK106, Bandelin electronic, Berlin, Germany). After that, the mixture was centrifuged at 15,682 g for 6 min to separate the supernatant (Eppendorf centrifuge 5415R, Hamburg, Germany). The experiment was performed in quadruplicate. Finally, the supernatant volume was adjusted to 5 mL with the solvent and filtrated through a 0.20 µm membrane (regenerated cellulose, Phenomenex, Torrance, California, USA).

2.8 Lycopene and β-carotene

Lycopene and β-carotene concentrations were measured by using High Performance Liquid Chromatography (HPLC, Agilent 1260-series, Waldbronn, Germany) according to the method described by Olives et al. (2006) which was modified by Panjai et al. (2017). The HPLC was equipped with a reverse-phase system with an autosampler and a Eurospher100-C18 column (250 x 4 mm, 5 µm packing material, KNAUER Wissenschaftliche Geräte GmbH, Germany). The mobile phases used were methanol/ACN (PubChem CID: 6342) (90/10 v/v) with triethylamine (TEA) (PubChem CID: 8471) 9 µM at flowrate of 0.5 mL min-1. Detection was achieved at 475 nm at 30 °C column temperature. The results were analysed by using ChemStation software (Agilent, Waldbronn, Germany). Lycopene (PubChem CID: 446925) and β-carotene (PubChem CID: 5280489) were used as the standards for a linear calibration curve between 0.02 and 20 µg mL^{-1}. Lycopene and β-carotene were expressed as milligram per kilogram dry mass.

2.9 Total phenolic concentration

Total phenolic content was determined by using the Folin-Ciocalteu method as described by Chen et al., (2015) with slight modifications by Panjai et al. (2017). In brief, a volume of 0.2 mL of the methanolic extract was adjusted to 0.5 mL with water and then freshly diluted with 0.5 mL of 1:10 Folin-Ciocalteu reagent. The mixture was then incubated at room temperature for 4 minutes prior to the addition of 1.5 mL of saturated sodium carbonate (PubChem CID: 516892) solution (75 g L^{-1}) and was further incubated for 120 min. The absorbance of the mixture was measured at 760 nm by using a spectrophotometer (Lambda 35 UV/Vis spectrophotometer, PerkinElmer, Boston, Massachusetts, USA) and the respective solvent was used as blank. Gallic acid (PubChem CID: 370) was used as the standard for a linear calibration curve between 50 and 500 mg L^{-1}. The total phenolics content was expressed as milligram of gallic acid equivalents (mg GAE g^{-1}) dry mass.

2.10 Total flavonoid concentration

Total flavonoid content was determined by using the aluminum chloride colorimetric method described previously (Chen et al., 2015) with slight modifications by Panjai et al. (2017). Briefly, a known volume (1 mL) of the methanolic extract was added to 0.1 mL of 5 % sodium nitrite (PubChem CID: 23668193) solution. The mixture was allowed to incubate for 6 min, then 0.1 mL of 10 % aluminum chloride (PubChem CID: 24012) solution was added and the mixture was further incubated for 5 min, prior to adding 0.1 mL of 1 M sodium hydroxide (PubChem CID: 14798) and was vortexed for 10 s. After adding 1.7 mL of water and 30 min of incubation, the absorbance was measured at 510 nm using a spectrophotometer (Lambda 35 UV/Vis spectrophotometer, PerkinElmer, Boston, Massachusetts, USA). The respective solvent was used as blank. Quercetin (PubChem CID: 5280804) was used as the standard for a linear standard curve between 100 and 1000 mg L^{-1}. The total flavonoids content was expressed as milligram of quercetin equivalents (mg QAE g^{-1}) dry mass.

2.11 Statistical analysis

Statistical analyses were performed by using SPSS 22.0 (IBM statistics version 22.0, Armond, NY, USA). Analysed data is expressed as means ± SE. One-way analysis of variance (ANOVA) was used and a Tukey HSD test was conducted to establish the differences among mean values. The threshold *p*-value chosen for statistical significance was $p \leq 0.05$. Graphs were generated by using SigmaPlot version 12.0 (Systat Software, Inc.) and the R package ggplot2 (Wickham, 2016)

3. Results and discussion

In order to investigate effective post-harvest measures to accelerate tomato fruit development, we applied continuous red light as a proven beneficial treatment (Panjai et al. 2017), with the aim to investigate metabolic and spectral changes of the outer epicarp and mesocarp and inner endocarp parts of tomatoes.

3.1 Chlorophyll content indicator, SFR_R

The color changes in fruit, indicated by SFR_R (Fig 1), was significantly affected by continuous red light irradiation. SFR_R of fruit treated with continuous red light for 21 days sharply decreased from DAH (day after harvest) 5 to DAH9 while fruit treated with red light for 12 hours and control fruit showed rapid decreases from DAH9 to DAH11 and DAH11 to DAH13, respectively. Furthermore, there was a gradual decrease in SFR_R in fruit treated with red light for 15 days and continuous red light. Moreover, the significantly lowest SFR_R value was recorded for fruit treated with continuous red light from DAH5 onwards. The fruit color, besides being a parameter decisive for consumer purchase, is also an indicator of the amount of pigment compounds (particularly lycopene) present in the fruit (Ana et al., 2014).

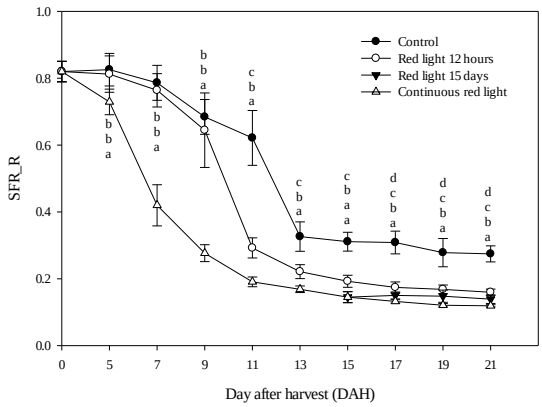

Figure 1. SFR_R of control tomatoes (closed circle) and tomatoes treated with red light for 12 hours (open circle), for 15 days (closed triangle) and continuously (open triangle) on harvest day (0) and 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after harvesting. Means ± SE, n = 5 fruit, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$)

3.2 External fruit color

The a* values and a*/b* of fruit treated with continuous red light noticeably increased between the second to third measurement day (DAH5-9) (Figs. 2A and 2B). This increase was seen in the other treatments as well, though at late measurement days

(DAH7-11 for fruit treated with red light for 12 hours and DAH9-13 for control fruit). Moreover, significantly highest values of a* and a*/b* were recorded for fruit treated with continuous red light from DAH5 to DAH13 compared to fruit treated with red light for 12 hours and control. As in this study, the a*/b* ratio of tomato surface color has been previously used as effective, reliable reference parameter for red color development in tomatoes (Arias et al., 2000). However, in our study, spectral information of reflected light pattern (of external fruit skin) revealed more detailed insight into the accelerated chlorophyll breakdown than the classical a*b* ratio being even more reliable than color assessment by eye, as shown previously (Kuska et al., 2018).

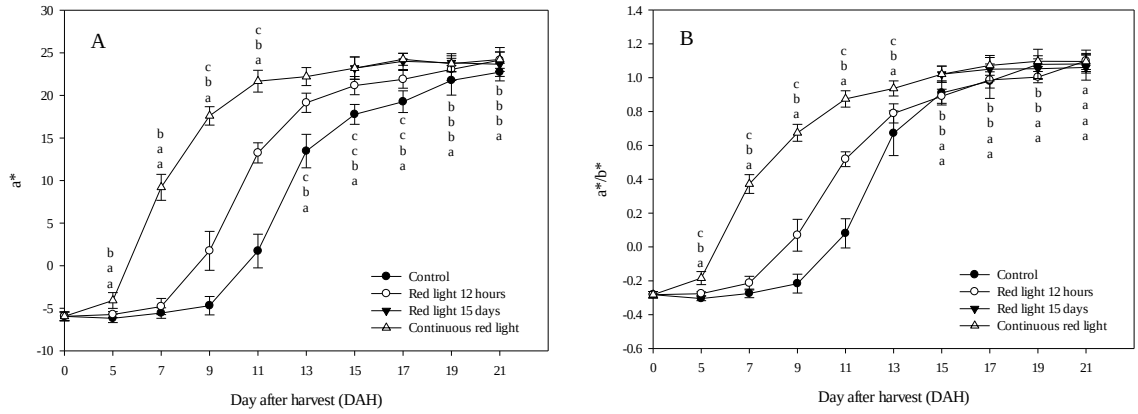

Figure 2. Values of (A) a* and (B) a*/b* of control tomatoes (closed circle) and tomatoes treated with red light for 12 hours (open circle), for 15 days (closed triangle) and continuously (open triangle) on harvest day (0) and 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after harvesting. Means ± SE, n = 5 fruit, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$)

Outer layer color development of fruits varied as an effect of treatment with different light regimes (Fig 3). The share of red light (650 – 700 nm) reflected by all tomato fruits increased gradually within 15 days, while the share of reflected green light (530 – 560 nm) decreased. The fastest shift from green to red was observed in tomatoes treated with continuous red light for 15 days, with virtually no difference to fully red fruits at day 11. Two days later, fruits treated with red light for twelve hours per day showed the same reflection pattern. Fifteen days after continuous red light treatment induction, tomatoes of all groups were identified as red with the bare eye, while the

reflectance sensor was still able to detect slight differences in untreated control tomatoes as compared to the other groups (Figure 3, Day 15).

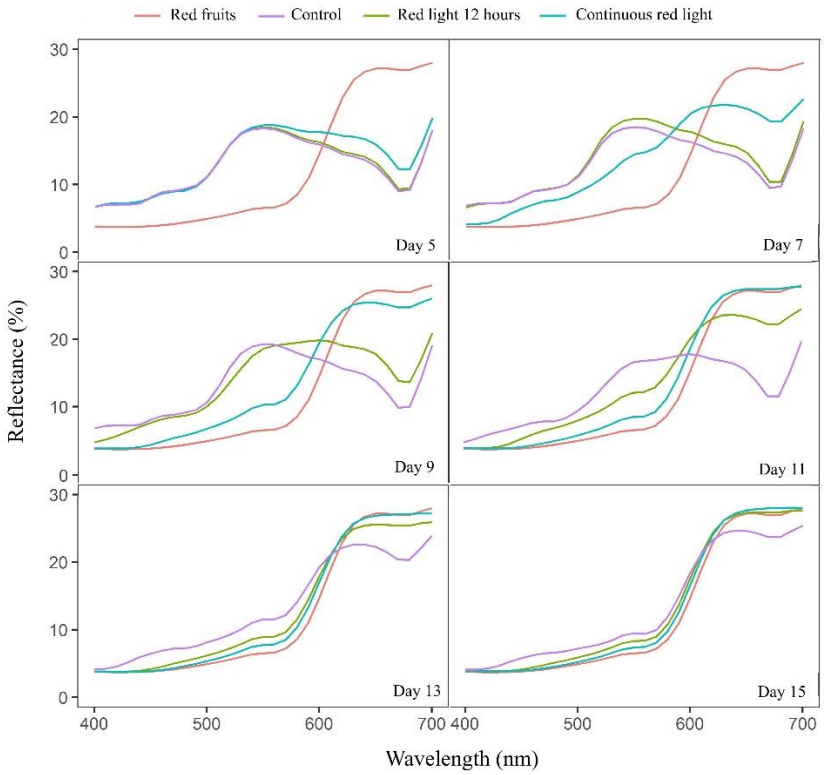

Figure 3. Spectral reflectance of tomato fruits treated with red light for 12 h per day (green line), continuous red light (blue line), or no additional light (purple line) on days 5 – 15 after treatment initiation. Spectral reflectance of ripe fruits added as reference (red line). Displayed spectra are means (n = 15).

This well-known chlorophyll breakdown during ripening affects reflectance especially at 680 nm, one of the main wavelengths of chlorophyll absorbance (Gitelson et al., 2006; Watada et al., 1976). As major changes in reflection patterns during fruit ripening were observed at 530 nm and 680 nm respectively, the green/red – ratio is sufficient to observe the shift in color. As reflection at 530 nm decreases and reflection at 680 nm increases during ripening, the quotient of both values can serve as a very sensitive indicator for tomato ripening, while full-range reflectance patterns reveal further insight into other structural changes (Zhu et al., 2015), such as shown for slightly different light applications in our study. Additionally, during the ripening process, it was evident that contents of other compounds accumulated in the range of 500 to 600 nm absorbance,

such as anthocyanin and carotene (Kuska et al., 2018), indicating an advanced development due to continuous red light compared with controls and the other red light applications. The ripening process has also been described through reduced concentrations of compounds that absorb above 600 nm, e.g. chlorophyll (Zhu et al., 2015).

3.3 Lycopene concentration

The content of lycopene in the outer part was higher than in the inner part of tomato fruit exposed to continuous red light (Fig 4). Lycopene concentration of the outer and inner part of fruit treated with continuous red light drastically increased from DAH5 to DAH7 in all treatments, even though this increase was most pronounced in fruits treated with continuous red light. Continuous red light led to a 2.84-fold higher lycopene concentration on DAH21 in the outer part of the fruit when compared to the inner part. This is in line with Sharma and Le Maguer (1996), observing that most of the lycopene content was associated with the skin and water insoluble fraction of the tomato pulp. About 80-90% of the total carotenoid content consists of lycopene, the red color of tomato (Shi and Le, 2000). George et al. (2004) studied antioxidant components in 12 field-grown tomato genotypes and reported that on average, the tomato epidermis had 2.5 times higher lycopene levels than the pulp. This might be due to the fact that the lycopene pigment is mostly associated to the skin and is also an insoluble fiber portion of tomato fruit (Toor and Savage, 2005).

3.4 ß-carotene concentration

β-carotene concentration of fruit treated with continuous red light was significantly different during storage time compared to the other treatments (Fig 5). Concentration of β-carotene was highest on DAH11 for the outer part of fruit treated with continuous red light as well as the inner part. After this peak, β-carotene concentration sharply decreased in all treatments. Interestingly, β-carotene concentration of the outer part subjected to continuous red light was 1.76-fold higher compared to the inner part. These results are also consistent with the fact that the tomato epidermis is rich in carotenoids (Toor and Savage, 2005).

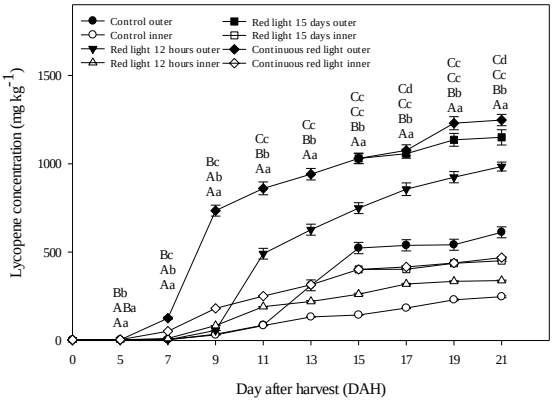

Figure 4. Lycopene concentration of the outer and the inner part of control tomatoes (closed and open circle), tomatoes treated with red light for 12 hours (closed and open triangle), for 15 days (closed and open square) and continuously (closed and open rhombus) on harvest day (0) and 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after harvesting. Means ± SE, n = 5 fruit, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

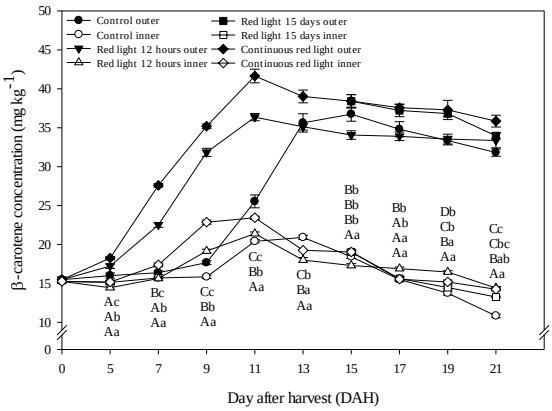

Figure 5. β-carotene concentration of the outer and the inner part of control tomatoes (closed and open circle), tomatoes treated with red light for 12 hours (closed and open triangle), for 15 days (closed and open square) and continuously (closed and open rhombus) on harvest day (0) and 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after harvesting. Means ± SE, n = 5 fruit, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.5 Total phenolic concentration

The total phenolic concentration in the outer and inner parts of fruit exposed to continuous red light sharply increased already from DAH0 until the end of the storage time (Fig 6). Furthermore, significantly highest values of total phenolic concentration were recorded for fruit treated with continuous red light throughout the experimental time compared to tomatoes treated with red light for 12 hours and control. Phenolic concentration of the outer part subjected to continuous red light was1.12-fold higher compared to the inner part on DAH21. Similar developments have been reported by George et al. (2004), where the phenolic content in the skin of different field-grown tomato genotypes ranged from 10–40 mg catechin equivalents/100 g, whereas the phenolic content ranged from 9–27 mg catechin equivalents/100 g in the pulp. In addition, the tomato skin had significant amounts of phenolics content (George et al., 2004). Furthermore, the phenolic content was found to be higher in tomato epidermis than in seeds and in the columella of different cultivars such as ‘Sindhu’ and ‘Shalimar’, which is also true for ‘Lyterno’ in this study (Chandra and Ramalingam, 2011).

3.6 Total flavonoid concentration

Total flavonoid concentration of the outer and inner part of fruit treated with continuous red light drastically increased during storage time (Fig 7). In addition, total flavonoid concentration of the inner part of fruit treated with red light for 15 days was not significantly different on DAH11 and DAH13 compared to tomatoes treated with red light for 12 hours. However, in both the outer and inner part of tomatoes, continuous red light stimulated total flavonoid concentration as those fruit showed significantly higher concentrations during storage time compared to the other treatments. At the end of the storage period, flavonoid concentration in the outer part was 1.45-fold higher compared to the inner part of the fruit. Similar to our findings, Stewart et al. (2000) reported that the majority of the flavonols in tomatoes are present in the epidermis. In addition, this study also identified the seed fraction of tomatoes as an important reservoir of phenolics. In our study, seeds could have influence flavonoid content, as they were included in the pulp.

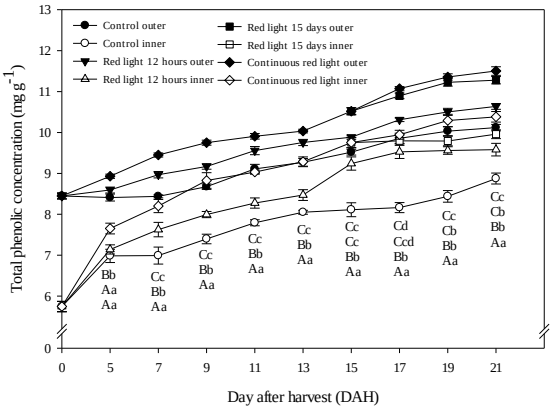

Figure 6. Total phenolic concentration of the outer and the inner part of control tomatoes (closed and open circle), tomatoes treated with red light for 12 hours (closed and open triangle), for 15 days (closed and open square) and continuously (closed and open rhombus) on harvest day (0) and 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after harvesting. Means ± SE, n = 5 fruit, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

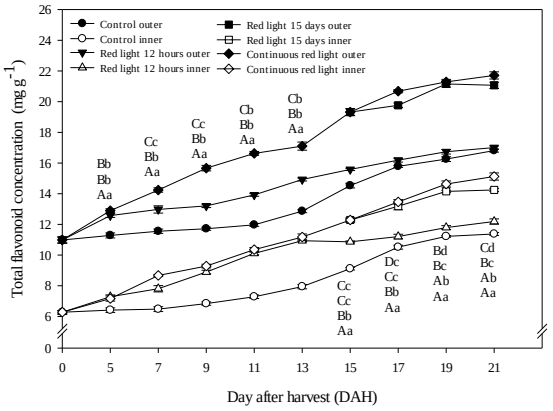

Figure 7. Total flavonoid concentration of the outer and the inner part of control tomatoes (closed and open circle), tomatoes treated with red light for 12 hours (closed and open triangle), for 15 days (closed and open square) and continuously (closed and open rhombus) on harvest day (0) and 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after harvesting. Means ± SE, n = 5 fruit, different letters within each day indicate significant differences between the treatments (ANOVA, $p \leq 0.05$).

3.7 Correlation between non-destructive indices and effective pigment concentrations

Changes in the a*/b* ratios were highly related (r^2=0.9583) to changes in the SFR_R value of fruit from all treatments during 21 days of storage time (Fig. 8). The higher the a*/b* ratio, the lower was the SFR_R value. Furthermore, there was a positive correlation between a*/b* and lycopene concentration in the outer (r^2=0.8461) and inner (r^2=0.8391) part of the fruit (Figs. 9A and B), while a highly negative correlation between SFR_R and lycopene concentration in the outer (r^2 = 0.9635) and inner (r^2 = 0.9344) part of the fruit was found (Figs. 9C and 9D). In addition, the relationship between a*/b*and SFR_R, and β-carotene of the outer part and the inner part is shown in Fig 10A, 10B, 10C and 10D. The correlation between variables indicates that a*/b*(r^2=0.0016) and SFR_R (r^2=0.0226) are not a reliable parameter to predict β-carotene concentration of the inner part of tomato. In contrast, there was a positive correlation between a*/b* (r^2=0.8211) and β-carotene concentration, while a negative correlation between SFR_R and β-carotene concentration (r^2=0.8552), in the outer part of tomato fruits. Obviously, spectral accessed estimations of the epicarp showed a better correlation with than β-carotene concentration than inner parts. Changes in the a*/b* ratio were highly related to changes in the SFR_R value of fruit from all treatments during 21 days of storage time. This may be explained by the fact that the skin is rich in pigment compounds, namely carotenoids represented by lycopene (80 to 90 %). Most approaches are very similar and describe tomato color development as an exponential process converting a green pigment complex into a red pigment complex. Furthermore, recent studies have shown that the cultivar and ripening stage of tomatoes might also affect their levels of lycopene and other antioxidants (Abushita et al., 2000; Gomez et al., 2001; Thompson et al., 2000). These factors could account for the variation in the lycopene levels reported in different studies. The red color of tomatoes is mainly due to the presence of lycopene (Sharma and Le, 1996). However, no significant differences were observed in the lycopene content of the seed and pulp fractions in the three cultivars (Tradiro, Flavourine and Excell). In the present study, we suggest that i) continuous red light positively affects compound contents in tomato skin and pulp, as well as that ii) the variation in fruit redness was referred to different lycopene content in the skin and that it can be reliably estimated by a*/b* values and SFR_R measurements of the skin.

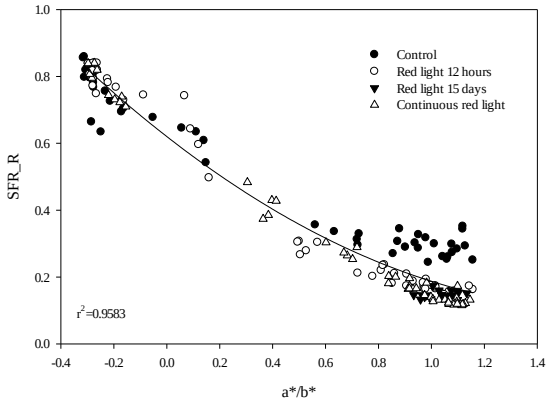

Figure 8. Correlation between a*/b* and SFR_R of control (closed circle) tomatoes, tomatoes treated with red light for 12 hours (open circle), red light for 15 days (closed triangle) and continuous red light (open triangle). The solid line indicates curve fitting. Equation and r^2 for the regression lines (all data) are given

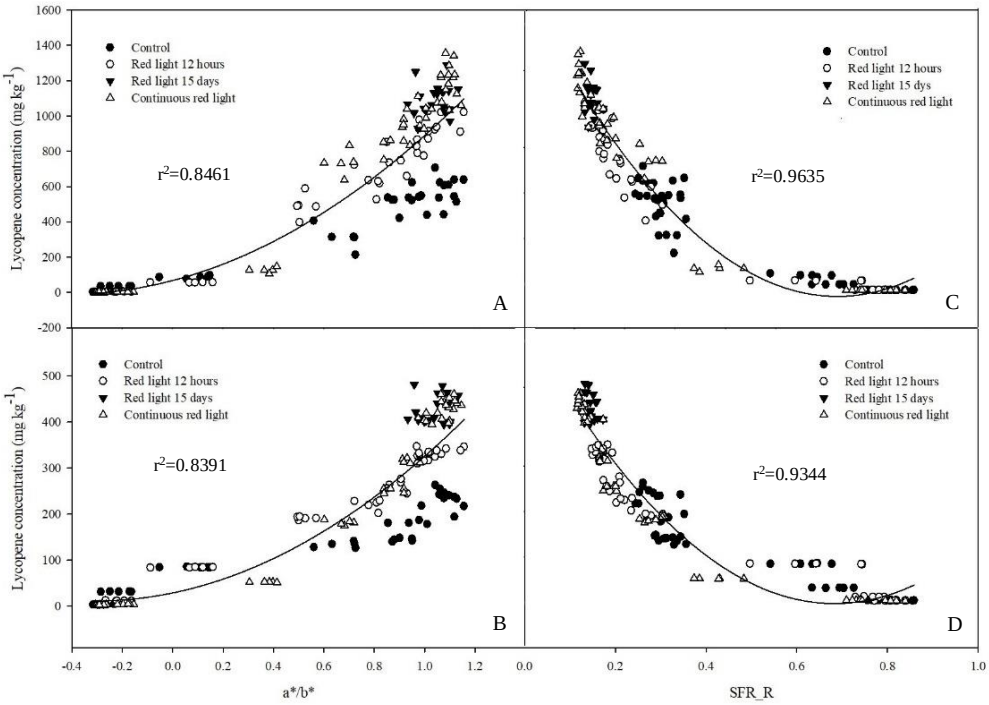

Figure 9. Correlation between a*/b* and Lycopene concentration of (A) the outer part, (B) the inner part, SFR_R and Lycopene concentration of (C) the outer part and (D) the inner part of control (closed circle) tomatoes, tomatoes treated with red light for 12 hours (open circle), red light for 15 days (closed triangle) and continuous red light (open triangle). The solid line indicates curve fitting. Equation and r^2 for the regression lines (all data) are given

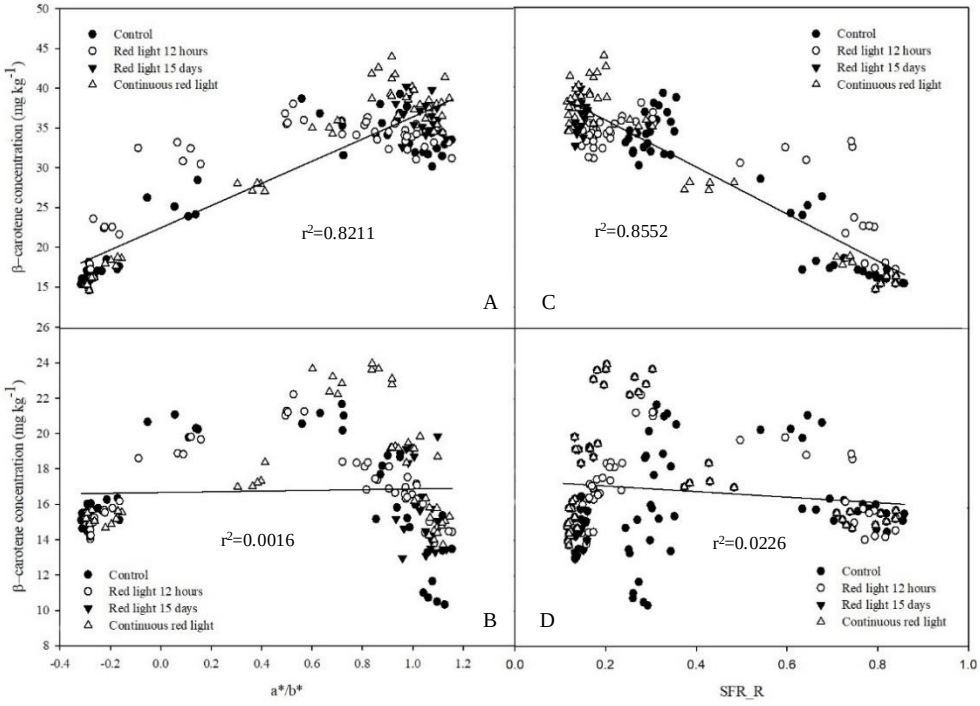

Figure 10. Correlation between a*/b* and β-carotene concentration of the (A) outer part, (B) the inner part, SFR_R and β-carotene concentration of (C) the outer part and (D) the inner part of control (closed circle) tomatoes, tomatoes treated with red light for 12 hours (open circle), red light for 15 days (closed triangle) and continuous red light (open triangle). The solid line indicates curve fitting. Equation and r^2 for the regression lines (all data) are given

4. Conclusion

To our knowledge, this is the first time that spectral measurements (SFR_R, a* values, a*/b*) were correlated with lycopene concentration of the inner and outer part of tomato fruit radiated with red light. Here, significantly higher levels of all analysed parameters were found in the outer part of the fruit compared to the inner part. Besides, this study suggests that the outer and inner parts of tomato are a very rich source of beneficial compounds. Our results also demonstrate that continuous red light irradiation positively affects color development of both the outer and inner parts of tomatoes, decreased SFR_R and a* values, whereas a*/b*, lycopene, β-carotene, total phenolic and total flavonoid concentration increased. In order to improve post-harvest storage and to

provide beneficial food both increased levels of valuable compounds, red light application might be considered.

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E Summary and conclusion

The main objective of the present work was to evaluate the optimal exposure time and effects of red light and short periods of daily ultraviolet (UV) on the post-harvest quality of green tomatoes during ripening. However, so far, little information is available about the effect of a combination of red light, and UV comparison to single red light irradiation and darkness schemes on the quality of outer and inner parts of immature green tomatoes during the ripening process. Therefore, experiments were conducted in a climate chamber equipped with red LED and UV tubes to evaluate the optimal exposure time and effects of red light and short periods of daily ultraviolet (UV) on the post-harvest quality of green tomatoes. In order to reach the objectives, simple chlorophyll fluorescence ratio, lycopene, β-carotene, total phenolic concentration, total flavonoid concentration, as well as hydrophilic and lipophilic antioxidant activity were monitored during the storage period.

The results ascertained in the single chapters:

1. Daily light treatment with **red light** as well as a **combination of red light with UV** was sufficient to **trigger full tomato ripening within 10 d after harvesting**, whereas non-treated fruits needed five more days to ripen to a stage where fruit can be sold. Red light and red light combined with UV prompted a faster chlorophyll breakdown, as shown by the NDVI results in this experiment. In addition, red light treatment of tomatoes enhances lycopene accumulation with minimal effects on the total soluble solid during 20 d of post-harvest storage. This indicates that red light is a **regulator of carotenoid synthesis** and accumulation in tomatoes during post-harvest storage. It can also be concluded that red light on its own was sufficient enough to induce similar concentrations of secondary metabolites as when combined with UV radiation, representing an important factor for tomato growers, as they do not necessarily have to install (costly) UV lamps in addition to LEDs.

2. **Continuous red light exposure** during post-harvest significantly increased secondary metabolites and antioxidants activity, which are among of the main important parameters in consumer preference. In addition, intermittent radiation does not seem to achieve similar effects in ripening speed and antioxidant capacity of tomatoes. Tomatoes exposed to continuous red light accumulated **significantly more lycopene and β-carotene, total flavonoid, phenolic concentration, HAA and LAA** during storage time in comparison with the control tomatoes and tomatoes treated with red light for a lesser amount of time. Moreover, **changes in firmness** throughout the post-harvest period were significantly affected by red light radiation and fruit treated with red light irradiation for 24 h showed the fastest softening during the storage period.
3. **The inner and outer parts of tomato** are a wealthy source of antioxidant compounds, and each part increases in the amount of all major antioxidants after red light irradiation in the full red ripening stage. Green stage-1 tomatoes were harvested and treated daily with red light for 12 hours per day, for 15 days (followed by storage in darkness for additional 6 days) or were continuously radiated with red light for 21 days. Control untreated tomatoes were kept in the dark for the same period. Color parameters of the outer part were strongly influenced by the application of continuous red light. Our results also demonstrate that **continuous red light irradiation positively affects color development of both the outer and inner parts of tomatoes**, decreased SFR_R and a* values, whereas a*/b*, lycopene, β-carotene, total phenolic and total flavonoid concentration increased. Here, significantly higher levels of all analyzed parameters were found in the outer part of the fruit compared to the inner part. This study suggests that **red light radiation, accelerates the color development by directly linking between skin color and lycopene synthesis in the outer and inner fruit tissue.**

The present study was carried out to investigate the preservation of the post-harvest quality of mature green tomatoes by applying light and comparing it to the tomatoes in darkness. Mature green stage 1 tomatoes were selected for this study because they can ripen even after being detached from the vines, and are the appropriate stage for

commercial purposes. It can be concluded from this study that daily red light, as well as a combination of red light with UV, are suitable treatments for tomatoes to enhance color and firmness by stimulating accumulation of carotenoids and antioxidative compounds during post-harvest storage and triggering full tomato ripening. Furthermore, tomato skin color (as measured by the Hunter a*/b* ratio) reflects epicarp lycopene and β-carotene contents.

Based on the finding of this study, the results demonstrated that post-harvest red light irradiation has a profound effect on carotenoid and secondary metabolites synthesis. This implies that red light treatment might be a potential tool to regulate the carotenoid metabolic pathway in climacteric fruits to modify color phenotypes and the contents of the major pigments such as lycopene and carotenes, which are increasingly recognized as essential phytonutrients with a diverse range of health benefits.

As far as the effects of lights are concerned, the data available are rare. Unfortunately, this study is limited; however, it gives a general, but defined idea of the trends of secondary metabolite synthesis during tomato ripening after red light stimulation. Thus, it would be worthwhile examining other patterns of delivering the red light dose, e.g., by fractionating the dose and providing reduced doses at fixed intervals of time. Moreover, further work would be needed to gain insight into the mechanism of red light irradiation on increased secondary metabolites and enhanced antioxidant activity, in order to also understand the effect of red light irradiation on the involvement of the major genes in secondary metabolites pathways in post-harvest tomato ripening.

In summary, the results obtained in our studies demonstrate the underexploited potential of light quality to increase the ripening speed of green stage 1 tomatoes. In order to improve ripening conditions of the post-harvest production process, red light treatment of immature green tomatoes might avoid workload peaks during harvesting, might improve efficient tomato storage in proper storage facilities, and thus, might help to overcome gluts of low pricing and disposable products. It is evident that a better understanding and technological advancement of the regulation of carotenoid metabolism in tomato hold enormous promise for satisfying both agricultural needs and scientific interests. More efforts should be made to facilitate the manipulation and improvement of agronomical and economical quality in tomato crops by elucidating the regulatory

mechanisms of the carotenoid metabolism network. The need to reduce post-harvest losses is therefore of paramount importance.

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